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THE MINERAL CONTENT OF GRAIN

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DURING the past ten years numerous fairly complete analyses of the mineral content of whole grains have been made at the Utah Experiment Station. A summary of these results is of considerable interest for a number of reasons: (1) In the case of wheat, oats, and barley they represent the averages of many determinations: Wheat 1755 samples, oats 1205 samples, barley 110 samples, and corn 75 samples. (2) These grains were grown under both irrigated and dry-farm conditions. (3) They were grown on soils having a wide variation in composition. The soils varied from the so-called "alkali-free" to "alkali soils" and from the highly calcareous to those in which calcium was present only in moderate quantities. (4) The sulfur of the soil varied greatly. Some of the soils were highly impregnated with gypsum while others contained only small quantities of sulfur. (5) The wheat represents various varieties grown under similar conditions; consequently, we have a crude measure of the effect of irrigation, soil, and variety on the mineral content. (6) The determinations of ash, calcium, magnesium, potassium, phosphorus, sulfur, and iron were all made by modern methods of analysis, usually those approved by the Association of Official Agricultural Chemists (6). In each table is given the maximum, the average, and the minimum percentage; consequently, we have the extremes in variation.

TABLE I

PERCENTAGE ASH IN OATS, BARLEY, WHEAT, AND CORN GROWN UNDER VARYING CONDITIONS

	Oats	Barley	Wheat	Corn
Maximum	4.87	3.71	2.94	1.79
Average	4.05	3.00	1.85	1.73
Minimum	1.68	2.08	1.35	1.65

[1] Publication authorized by Director, December 8, 1928.

There is a variation of 290, 178, and 218 per cent between the maximum and minimum ash content of oats, barley, and wheat, respectively. The small variation in the case of corn is due to the fact that all the samples analyzed were grown on the same soil and represent only one variety. It is evident from these results that animals kept on the same rations, but with grains coming from different sources, may be receiving a great variation in ash. The probable extreme variation shown by our work which may be attributed, in the case of wheat, to irrigation water is 46 per cent, to variety 31 per cent, and to soil 118 per cent.

These average results for oats are considerably higher than those reported by Armsby (1), Hopkins (3), König (4), or Sherman (7). The Utah-grown barley carries an ash content almost the same as those reported by Hopkins. There is a remarkable agreement in the average ash content of the Utah-grown wheat and that reported by König and the 20-year averages for the Rothamsted work (5).

TABLE II

PERCENTAGE CALCIUM IN OATS, WHEAT, BARLEY, AND CORN GROWN UNDER VARYING CONDITIONS

	Oats	Wheat	Barley	Corn
Maximum	0.190	0.296	0.150	0.180
Average	0.117	0.090	0.086	0.150
Minimum	0.019	0.028	0.029	0.130

So far as calcium is concerned, there is a much wider spread in variation in the case of wheat than any of the other grains. Considered from the standpoint of average, oats are richer in calcium than any of the other grains; however, a few wheats contained more calcium than any of the oats.

The average calcium content of these grains is about double that reported as the 20-year average for the Rothamsted work, and they are considerably higher than those reported by Sherman. It is most probable that this would have a profound influence in the nutrition of farm animals which may be kept on a ration consisting primarily of grains. The part it plays in human nutrition where the extensive use of highly-milled grains enters will be known only after determinations have been made on the milled and natural grain.

Wheat grown in some localities was found to carry six times the calcium of wheat grown in other localities. Wheat raised with 67.5 inches of irrigation water contained 2.6 times as much calcium as did similar wheat

grown on similar soil without irrigation water. Some varieties of wheat even though grown under similar conditions contained twice the calcium contained in other varieties. Similar variations were found to exist in the case of the oats and barley. Many of these grains were grown on highly calcareous soil and often with irrigation water, which are the reasons for the high calcium content of these grains.

TABLE III

PERCENTAGE MAGNESIUM IN WHEAT, OATS, BARLEY, AND CORN GROWN UNDER VARYING CONDITIONS

	Wheat	Oats	Barley	Corn
Maximum	0.276	0.278	0.273	0.220
Average	0.184	0.181	0.181	0.203
Minimum	0.131	0.141	0.147	0.190

The ash and total calcium varied with the different grains, but no such variation is found in the case of barley; in fact, the maximum and average are within experimental error. However, the variation within each specific grain is large.

The variation in magnesium content among different varieties amounted to 25 per cent and that due to irrigation 32 per cent. The average magnesium content of the various varieties of wheat grown at Nephi were 0.184 per cent, whereas the average of the Greenville-grown wheat was 0.188 per cent. The calcium content of the Nephi-grown wheat was 0.079 per cent and that of the Greenville-grown wheats 0.167 per cent; consequently, while the magnesium content of the grains from the two districts is not far different, the calcium content is twice as high in the Greenville-grown grain as it is in the Nephi-grown. This is due to two factors: (1) The greater calcium content of the Greenville soils and (2) the application of irrigation waters to some of the Greenville-grown grains.

The average magnesium content of all these grains is considerably higher than reported by other workers. However, the increase in magnesium is not so great as the increase in calcium. This is seen from an examination of the calcium-magnesium ratios. These ratios for the grains reported by König are: Wheat, 1:3.1; oats, 1:1.7; barley, 1:1.7. The calcium-magnesium ratios of the Utah-grown grains are: Wheat, 1:2; oats, 1:1.5; and barley, 1:2.1. These results establish three important factors: (1) There is a great variation in the magnesium content of grains,

wheat, oats, and barley, depending upon the composition of the soil on which they are grown; (2) the magnesium content of grains, wheat, oats, and barley, varies with the quantity of irrigation water used in their production; and (3) the average magnesium content of Utah-grown grains is higher than that usually reported from other districts.

The calcium and magnesium contents of the corn here reported is much higher than that reported by other workers. This, however, may not represent the general calcium and magnesium contents of Utah-grown corn, as the samples all came from one district.

TABLE IV

PERCENTAGE POTASSIUM IN WHEAT, OATS, BARLEY, AND CORN GROWN UNDER VARYING CONDITIONS

	Wheat	Oats	Barley	Corn
Maximum	0.960	1.000	0.923	0.410
Average	0.476	0.570	0.561	0.390
Minimum	0.307	0.418	0.389	0.370

The variation in potassium content of wheat of different varieties grown under the same conditions amounted to 51 per cent, the variation due to irrigation water 35 per cent, and the variation due to locality, primarily soil, to 28 per cent. The average potassium content of wheat, oats, and barley, as here reported, is not greatly different, but the variation between grains grown under different conditions is very great. We have an extreme variation between the maximum and minimum in wheat of 312 per cent, in oats of 239 per cent, and in barley of 237 per cent. The average potassium content of Utah-grown grains is higher than those reported for some districts and lower than those reported for others, but the difference is in no case so great as was found for calcium and magnesium.

TABLE V

PERCENTAGE OF PHOSPHORUS IN WHEAT, OATS, BARLEY, AND CORN GROWN UNDER VARYING CONDITIONS

	Wheat	Oats	Barley	Corn
Maximum	0.458	0.451	0.440	0.350
Average	0.331	0.364	0.351	0.334
Minimum	0.150	0.267	0.238	0.320

All the grains listed, except corn, show a wide spread in their phosphorus content, the extreme difference in the case of wheat amounting to over

300 per cent. All evidence points to the conclusion that there are at least three factors causing the variation—irrigation, soil, and variety of grain grown.

The average phosphorus contents of these grains are lower than the phosphorus reported by others. Consequently, there is a difference in the calcium-phosphorus ratio. The calcium-phosphorus ratios calculated from the great number of determinations reported by König are: Wheat, 1:8.9; oats, 1:4.4; barley, 1:4.4; corn, 1:12.8; whereas those for the Utah-grown grains are: wheat, 1:3.7; oats, 1:3.1; barley, 1:3.1; corn, 1:2.2. This greater proportion of calcium to phosphorus in the Utah-grown grains should make of them a more nearly balanced food than is the case with the grains proportionately higher in phosphorus. Whether this narrower ratio will be maintained in the highly-milled products can be answered only by further work.

TABLE VI

PERCENTAGE SULFUR IN WHEAT, OATS, AND BARLEY GROWN UNDER VARYING CONDITIONS

	Wheat	Oats	Barley
Maximum	0.287	0.294	0.241
Average	0.204	0.190	0.155
Minimum	0.120	0.115	0.082

The variation in sulfur content of wheat, which our work indicates can be attributed to irrigation water, is 48 per cent; to variety, 40 per cent; and to soil 30 per cent. The wheat showed a maximum variation of 139 per cent. The average sulfur content of wheat is 8 per cent greater than it is in oats and 32 per cent greater than it is in barley. The sulfur contents here reported are higher than those given in work referred to in this article, but this cannot be taken as an indication of a high sulfur content of Utah-grown grains as these determinations were made by the moist-fusion method, whereas most others were obtained by the ashing of the grains. This would result in a considerable loss of sulfur.

TABLE VII

PERCENTAGE IRON IN WHEAT, OATS, AND BARLEY GROWN UNDER VARYING CONDITIONS

	Wheat	Oats	Barley
Maximum	0.032	0.022	0.021
Average	0.005	0.007	0.006
Minimum	0.001	0.004	0.004

While the iron content of wheat, oats, and barley is not widely different, the difference which was found to exist between different samples grown under varying conditions was very great. We found that irrigation water, soil, and variety all contributed to a variation in the iron content of the various grains. These results are not far different in many cases from those reported by others, and what difference there is may be due to methods. We used the colormetric method of Berman (2) after the ash had been prepared by the Official Methods (6).

Summary

The ash, calcium, magnesium, potassium, phosphorus, sulfur, and iron contents of wheat, oats, barley, and corn are reported. The maximum, average, and minimum contents of each mineral for the various grains grown under varying conditions are given. Variation in the mineral content of different samples of each grain is great, the variation being due to the use of irrigation water, the nature of the soil on which the grain was grown, and the variety of grain. In the case of wheat, a crude measure of the effect of each of these factors is given.

The calcium, magnesium, and sulfur contents of these grains are higher than those usually reported for grains grown elsewhere. The phosphorus content is lower; consequently, the proportion of calcium to phosphorus is higher than usually reported. The question is raised whether these factors would not materially modify the nutritive value of grains grown on these highly fertile calcareous soils.

BIBLIOGRAPHY

1. Armsby, H. P., The Nutrition of Farm Animals. New York, 1917.
2. Berman, Lewis, A Rapid Method for the Determination of Iron in Small Quantities of Blood. *Jour. Biol. Chem.*, 1918. XXXV, 231–236.
3. Hopkins, C. G., Soil Fertility and Permanent Agriculture, Boston, 1910.
4. König, J., Der menschlichen Nahrungs und Genussmittel, II Band. Berlin. 1893.
5. Lawes, J. B., and Gilbert, J. H., On the Composition of the Ash of Wheat Grain and Wheat-Straw Grown at Rothamsted in Different Seasons and by Different Manures., *Jour. Chem. Soc.*, 1884, XLV, 305.
6. Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists. Washington, D. C. 1924.
7. Sherman, Henry C., Chemistry of Food and Nutrition. New York, 1926.

BLOOD FORMATION OF THE ALBINO RAT ON A STANDARD DIETARY RÉGIME*

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DURING October of 1927 we started an extensive investigation on the effect of vitamin deficiencies on hematopoietic function. In order to make comparisons with control animals of the same age and sex, we were obliged to secure data on our own stock colony, since we found the literature fragmentary and incomplete. Williamson and Ets (1) have studied the effect of age on the hemoglobin content of the albino rat, but show no data on erythrocytes, neither are any findings reported on the relation of sex to hematopoietic function. The results of Williamson (2) on the influence of age and sex on the hemoglobin content of the human are of interest but cannot be used by us as a basis of comparison in investigations involving the rat as the experimental animal. Furthermore, we found no information in the literature on the effect of fertility and lactation on hematopoietic function on an adequate diet.

Since 1921 we have had a pure strain of the albino rat secured from the Wistar Institute and for our breeding colony have used during the past 7 years a standard diet (referred to in previous publications as Stock Diet No. 1) (3) of the following composition:—whole wheat 27%; rolled oats 26%; yellow corn 25%; oil meal 15%; commercial casein 5%; cod liver oil 1%; NaCl 0.5%; $CaCO_3$ 0.5%; and a liberal supply of fresh whole milk daily. The data submitted in this communication were obtained on animals that received exclusively the above diet.

We are reporting in this paper on the influence of age and sex on the hemoglobin content and erythrocyte counts beginning with the 1st day of life and extending to the 340th day of the period of growth. In connection with this problem we have made 783 hemoglobin determinations and an equal number of erythrocyte counts. (Table I) Beginning with the 75th day we have also studied the influence of age and sex on the total leucocyte count of 17 animals (Table III). In addition, we have investigated the effect of fertility and lactation on hematopoietic function (Tables IV and V).

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TABLE I

NUMBER OF ANIMALS OF DIFFERENT AGES EMPLOYED FOR HEMOGLOBIN DETERMINATIONS AND ERYTHROCYTE COUNTS

Age (days)	*Male*		*Female*		*Both Sexes*	
	Number	Average weight of each animal (gm.)	Number	Average weight of each animal (gm.)	Number	Average weight of each animal (gm.)
1					18	4.18
3					18	5.33
5					21	6.80
7					18	9.33
9					18	12.11
11					18	14.10
13					18	17.00
14	11	23	6	21		
15	8	23	7	22		
16	9	24	18	22		
18	29	29	25	26		
19	16	28	14	28		
20	15	28	14	28		
21	15	35	15	31		
22	14	39	13	37		
23	12	37	22	40		
24	13	40	11	41		
26	11	44	16	45		
28	10	50	16	50		
30	19	54	17	53		
32	13	57	14	55		
34	6	51	17	57		
38	11	84	12	79		
44	11	101	13	98		
50	11	133	12	116		
63	14	170	10	128		
75	11	203	12	157		
83	4	215	20	160		
93	11	240	12	186		
123	11	276	9	205		
175	5	304	6	231		
200	6	334				
210			6	245		
278	11	275				
340	10	340				
Total	317		337		129	

Total number of determinations—783.

The technique employed is essentially that of Hart and co-workers (4). We performed peripheral bleedings by clipping the rat's tail with a scissors. Hemoglobin determinations were made according to the method of Newcomber (5) on a Bausch and Lomb colorimeter with a standardized colored glass disc, which served as a hemoglobinometer attachment. The accuracy of the colorimetric readings checked with Van Slyke and Neill's oxygen capacity method (6) to the extent of ± 3 per cent. For the erythrocyte and leucocyte counts we used a Levy hemocytometer with single Neubauer ruling. A number of control experiments have revealed no essential differences between results secured from peripheral and arterial blood. Our findings are presented in Charts 1 to 3 inclusive and Tables I to V inclusive. All of our animals made very good growth. The males, however, during the ages of 200 to 278 days, showed a decline in weight which they regained during the succeeding 60 days. The reason for this performance we cannot explain.

Hemoglobin Content and Erythrocyte Counts During the Nursing Period

1. *The Effect of Age.* Chart 1 shows that the erythrocytes increase throughout the nursing period from 2.8 to 5.0 millions per cubic millimeter of blood. The notable rise in the red blood corpuscles begins, however, on the 7th day of lactation. The concentration of hemoglobin increases slightly during the first 3 days but falls markedly during the subsequent 12 days of the suckling period, i. e. a drop from 10.0 to 6.0 grams per 100 cc. of blood. During the rest of the nursing period the hemoglobin curve is almost a straight line. From the day of weaning (23rd to 24th) the hemoglobin rises rapidly in the succeeding 5 days, viz. an increase of 33 per cent. Our results shown in Chart 1 represent 416 hemoglobin determinations and an equal number of erythrocyte counts; also 55 similar determinations covering the post-weaning period (24th to 29th day). Williamson and Ets (1) show, as an average of 25 determinations, a hemoglobin content in the rat, between the ages of 1 to 19 days, of 12.94 grams per 100 cc. of blood. Their figures are considerably higher than ours, even allowing for the greater concentration of hemoglobin in nurslings during the first half of the lactation period. Their average is also higher than that of Nicholas' (7) values for young of the albino rat at birth, namely, 11.05 grams per 100 cc. We are at present investigating whether such differences in the hemoglobin content of nursing young are attributable to differences in the maternal diet.

2. *The Effect of Sex.* Chart 1 shows that sex has practically no influence

on the erythrocyte counts between the 14th and 24th day of the nursing period. The effect of sex on the hemoglobin content of nurslings during the same period is only slight from the 17th day on, but is somewhat higher in the female between the 14th to the 17th day. No attempt has been made to differentiate the sexes up to the 15th day of lactation.

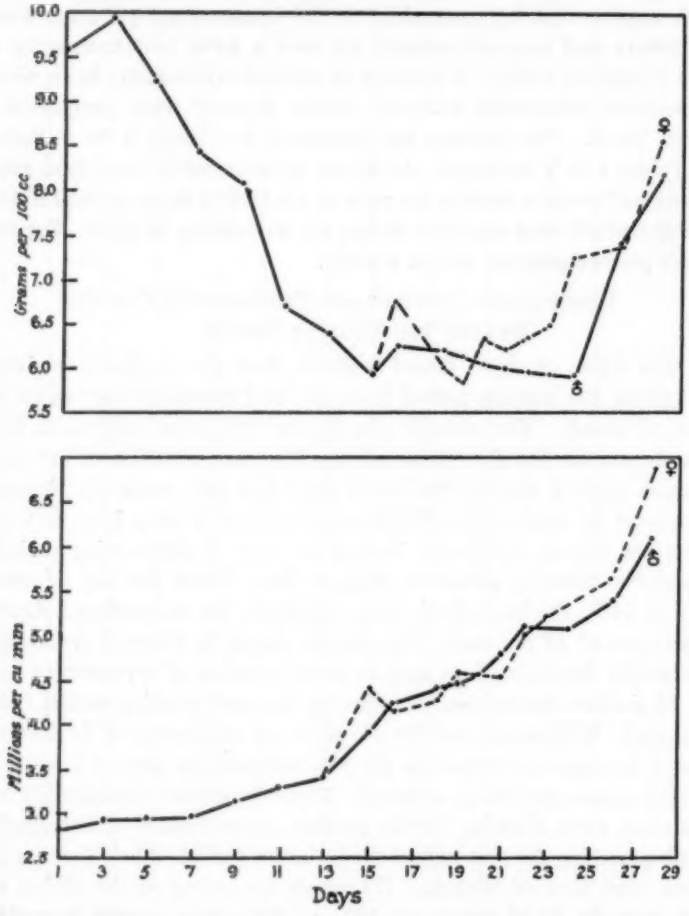

CHART 1. The influence of age and sex on the hemoglobin content and erythrocyte counts of the albino rat during the nursing period. (End of nursing period is the 24th day.)
Upper curves represent hemoglobin expressed as grams per 100 cc. of blood.
Lower curves represent erythrocytes expressed in millions per cubic millimeter of blood.

Hemoglobin Content and Erythrocyte Counts During the Period of Growth

1. *The Effect of Age.* For our work on hematopoietic function during the nursing period we have employed a series of different litters of increasing ages. In order to proceed later with studies on the effect of fertility and lactation on the hemoglobin content and erythrocyte counts, we selected 12 males and 12 females and followed the blood picture on the same animals throughout the period of growth, and later during gestation and lactation. For brevity we are reporting in Table II results of only two males and two females which are typical for the rest of the 20 animals. Our results are summarized and submitted graphically in Charts 2 and 3. The curves in these charts represent 312 hemoglobin determinations and an equal number of erythrocyte counts during the period of growth, beginning with the 30th day, of the 24 animals referred to above and in addition various groups of other animals ranging in age from 30 to 93 days. To these should be added 55 similar determinations covering the post-weaning period (24th to 29th day) shown in Chart 1. It is clear from Chart 2 that the first peak in the hemoglobin curve is reached on the 75th day (14.5 grams of hemoglobin per 100 cc. of blood) which is followed by a maintenance curve until the 120th day. From this point on up to the 180th day of life there is a continuous drop in hemoglobin concentration to 13.0 grams per 100 cc. From the 180th to the 280th day another rise in hemoglobin occurs to a maximum of 15.0 grams per 100 cc., and from then on to the termination of our experiment another decrease in hemoglobin takes place, so that on the 340th day the concentration is 12.83 grams per 100 cc. of blood. Our results are somewhat at variance with those of Williamson and Ets (1). They show a continuous rise in hemoglobin up to the 160th day, followed by a drop to the 250th day,—a maximum concentration of 15.51 and minimum concentration of 13.80 grams hemoglobin per 100 cc. of blood. Whether these differences are due to differences in diet we cannot say at present. We do know, however, that a synthetic diet containing an abundance of yeast (8) produces a different hematopoietic picture than encountered on stock diet No. 1.

2. *The Effect of Sex.* Chart 2 shows that the hemoglobin curve of the female follows very closely that of the male with the exception of the short period between the 75th and 90th day when the female shows one gram less hemoglobin per 100 cc. of blood. From Chart 3 it is evident that sex plays an insignificant role in determining the concentration of red blood corpuscles. The only variation in erythrocytes between the male and

TABLE II
THE EFFECT OF AGE ON HEMATOPOIETIC FUNCTION OF THE ALBINO RAT
(Peripheral bleeding on the same animal)

Animal number	Age (days)	Weight (grams)	Erythrocytes million per c. mm.	Hemoglobin grams per 100 cc.
♂101	18	33	3.72	6.07
	26	53	5.64	6.40
	37	85	8.96	9.90
	49	143	8.40	11.38
	75	230	9.84	13.52
	92	258	9.88	15.56
	125	292	9.04	16.11
	204	334	11.70	15.56
	277	272	8.88	16.11
	340	324	8.12	13.34
♀114	18	25	3.83	4.32
	23	37	5.80	7.50
	30	55	7.60	10.82
	38	75	7.28	10.56
	53	108	7.76	10.82
	76	147	8.44	16.66
	95	160	8.12	15.07
	125	179	9.40	13.71
	179	199	9.64	13.34
	210	208	9.60	14.58
♂104	18	33	3.52	5.50
	26	53	5.36	6.27
	37	70	5.20	8.18
	49	119	5.72	8.80
	75	212	9.16	13.52
	92	245	11.36	15.56
	125	275	9.56	15.07
	204	320	8.48	13.71
	277	256	8.48	15.56
	340	316	8.08	11.07
♀115	18	26	3.82	5.63
	23	39	5.44	5.19
	30	69	5.80	7.38
	38	96	8.00	9.72
	51	132	7.92	11.68
	74	164	9.00	17.28
	93	182	8.08	14.58
	123	196	8.72	17.28
	179	226	9.00	14.58
	210	244	9.40	15.07

female is encountered between the 75th and 120th day during which period the female shows a concentration of one-half to one million less red blood corpuscles per cubic millimeter. From the 30th to the 75th day and from the 120th to the 210th day the curves of the two sexes run parallel.

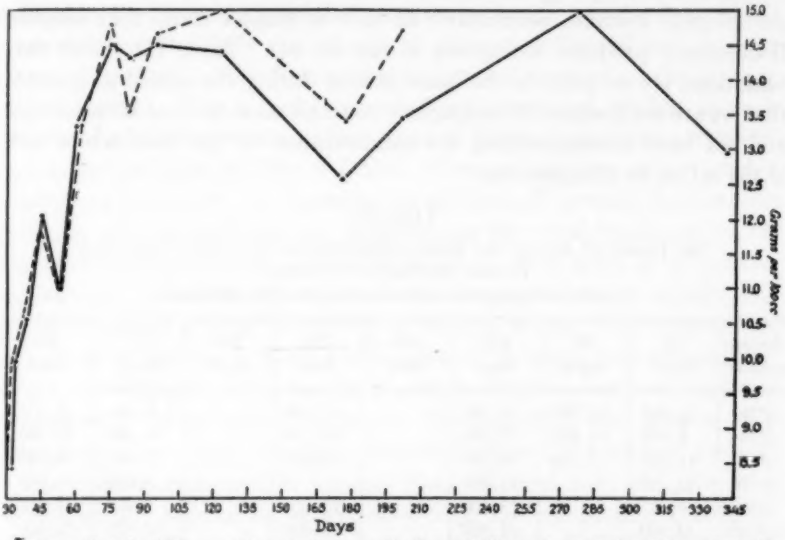

CHART 2. The influence of age and sex on the hemoglobin content of the albino rat during the period of growth.

Hemoglobin is expressed as grams per 100 cc. of blood.

Heavy lines represent the male; dotted lines the female.

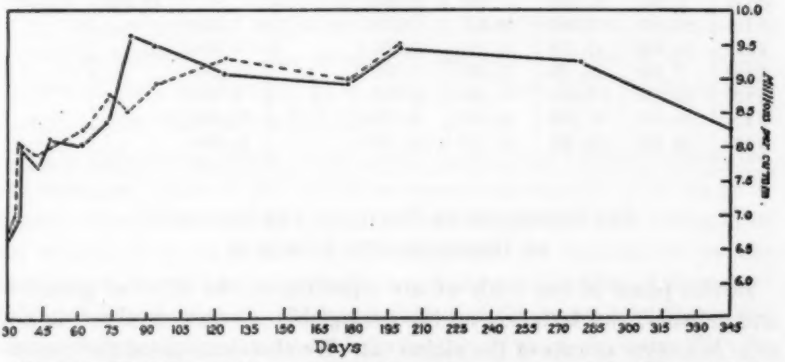

CHART 3. The influence of age and sex on the erythrocyte counts of the albino rat during the period of growth.

Erythrocytes are expressed in millions per cubic millimeter of blood.

Heavy lines represent the male; dotted lines the female.

The Influence of Age and Sex on the Total Leucocyte Count

We were particularly anxious to obtain information on this point on a standard dietary regime to serve as a basis for comparison with our pathological animals, particularly in view of Happ's claim that vitamin B-deficiency produces leucopenia in the rat (9). Table III shows that variations are so great in the same animal during the period of growth, that we are not justified in making any conclusions as to what influence age and sex have in determining the concentration of the total white cells in the rat on an adequate diet.

Table III

The Effect of Age on the Total Leucocyte Count of the Albino Rat During the Period of Growth
(Leucocyte figures represent number per cubic millimeter)

Animal number	75 days	90 days	125 days	180 days	200 days	210 days	275 days	340 days
♂101	12,025	12,500	11,700		11,350		9,400	8,350
♂104	5,850	13,450	17,050		10,450		10,500	11,350
♂105	14,425	7,850	12,500		10,050		9,650	8,750
♂107	13,700		12,350				8,000	15,500
♂108	9,675		13,300		12,600		9,400	14,000
♂111	14,600		11,950		12,000		10,050	14,100
♂117	11,000	13,400	15,300	9,350			12,050	
♂119	12,000	13,800	15,850	12,450			12,050	15,800
♂122	7,625	11,950	17,550	11,300			9,200	15,400
♂124	12,250	10,950	13,650	12,600			10,700	15,350
♂125	6,860	14,000	14,650	12,600			14,200	10,250
♀114	16,600	9,850	10,850	10,750		7,200		
♀115	10,100	15,350	11,250	10,000		7,500		
♀118	7,100	10,700	9,250	7,500		11,200		
♀120	13,150	9,300	13,200	12,000		9,600		
♀126	10,675	17,000	10,350	14,550		10,050		
♀127	10,250	16,300	13,150	14,550		11,050		

The Influence of Fertility and Lactation on Hematopoietic Function

In this phase of our work we are reporting on the effect of gestation and delivery, and lactation on the hemoglobin content, erythrocyte and total leucocyte counts of the albino rat. We also determined the concentration of the serum proteins by the method of the refractive index (10), using an Abbe refractometer. Since the blood had to be centrifuged, in

order to obtain serum for the determination of the refractive index, we had the opportunity of securing hematocrit readings at the same time. Such values are, therefore, also submitted.

1. *The Effect of Gestation and Delivery.* In this study we have not been interested in the early stages of pregnancy which can be determined accurately only by daily vaginal smears. Our work limited itself to later stages of gestation when unmistakable external signs of pregnancy are quite apparent. The animals were bled peripherally at mating, at such periods of gestation when the first external signs of pregnancy were detected, and on date of birth of litters. Four typical cases out of twelve studied are presented. An examination of Table IV reveals that neither gestation nor delivery has any influence on the total leucocyte count. The variation amongst the different individuals are so great as to warrant no definite conclusions. One fact stands out prominently, namely, the production of an anemia on the day of birth of young. This is to be expected, since delivery is always accompanied by slight and occasionally severe hemorrhages. This anemia is only temporary, however, and disappears with the advance of lactation. The surprising fact to us is that pregnancy, on our stock diet No. 1, should produce such a marked anemia, as has been encountered in animals 114, 115, and 118; also that the anemia should become so accentuated with the advance of gestation. We are at present, however, not finding an anemia during gestation on snythetic diets containing an abundance of wheat embryo or yeast as sources of the vitamin B complex (8). It is also evident from Table IV that during advanced stages of pregnancy there is an appreciable drop in the concentration of serum proteins, or in other words, the blood is less concentrated (10). The changes in the concentration of the serum proteins at birth are fluctuating, in some cases there being no appreciable change, and in others a marked rise.

2. *The Effect of Lactation.* Four typical cases out of twelve investigated are submitted. Table V shows that recovery from anemia found on birth of litters takes place with the advance of lactation and that the hemoglobin and erythrocytes reach the highest values at the termination of the nursing period when the young begin to partake considerably of the maternal diet. The changes in the concentration of the serum proteins in the mother during various stages of lactation are of such character that no definite correlations can be drawn. Lactation has no influence on the total leucocyte count.

TABLE IV

THE EFFECT OF GESTATION AND DELIVERY ON HEMATOPOIETIC FUNCTION OF THE ALBINO RAT

Female number	Period of gestation	Age days	Weight grams	Hemoglobin grams per 100 cc.	Erythrocytes million per c. mm.	Total leucocytes	Hematocrit	Serum protein per cent
114	At first mating	210	208	14.58	9.60	7,200		
	Incipient pregnancy	226	237	13.34	8.80	10,800	38	7.26
	Advanced pregnancy	232	265	8.61	7.72	9,400	34	6.51
	At birth of first litter	238	224	7.87	7.08	9,050	32	6.51
	Incipient pregnancy	269	226	15.56	9.76	8,400	37	6.98
	Advanced pregnancy	289	264	12.98	9.60	12,000	35	6.16
	At birth of second litter	294	238	11.38	8.16	8,650	32	7.15
115	At first mating	210	256	15.07	9.40	7,500		
	Incipient pregnancy	226	280	12.61	8.52	8,400	36	6.80
	Advanced pregnancy	234	329	8.80	7.28	14,350	32	5.81
	At birth of first litter	238	260	9.90	6.88	7,150	31	5.75
	Incipient pregnancy	282	292	13.34	9.60	12,400	36	7.38
	Advanced pregnancy	285	310	13.71	7.92	8,140	30	6.87
	At birth of second litter	289	260	12.30	7.88	10,150	29	6.45
118	At first mating	210	245	13.20	9.20	11,200		
	Incipient pregnancy	226	256	13.34	8.64	11,650	35	7.50
	Advanced pregnancy	234	280	8.18	7.40	7,700	28	6.11
	At birth of first litter	245	250	10.56	7.40	6,900	26	6.51
	Advanced pregnancy	269	292	13.71	6.76	7,000	28	5.52
	At birth of second litter	274	267	8.06	4.80	14,000	33	6.98
120	At first mating	212	227	15.56	8.88	9,600		
	Advanced pregnancy	232	272	12.61	7.72	9,300	36	6.33
	At birth of first litter	238	230	9.90	6.80	9,000	32	6.45
	Advanced pregnancy	282	326	11.93	6.40	11,700	30	5.35
	At birth of second litter	284	262	10.33	6.68	9,800	34	5.35

NOTE: "Incipient pregnancy" refers to first external signs of pregnancy.

TABLE V

THE EFFECT OF LACTATION ON HEMATOPOIETIC

	At birth of second litter										

NOTE: "Incipient pregnancy" refers to first external signs of pregnancy.

TABLE V

THE EFFECT OF LACTATION ON HEMATOPOIETIC FUNCTION OF THE ALBINO RAT

Animal number	Period of lactation	Litter	Age of mother (days)	Weight of mother (grams)	Weight of litter (grams)	Hemoglobin grams per 100 cc.	Erythrocytes millions per c. mm.	Total leucocytes	Hematocrit	Serum protein (per cent)
114	1st day	1st	238	224	34 (6)*	7.87	7.08	9,050	32	6.51
	13th "		249	228	150	9.72	6.80	7,150	24	6.33
	22nd "		260	220	298	11.93	7.20	8,000	33	6.51
	1st "	2nd	294	238	35 (7)*	11.38	8.16	8,650	32	7.15
	15th "		309	243	218	12.61	7.88	10,050	31	7.21
	24th "		318	239	338	14.58	7.80	8,900	36	7.44
115	1st "	1st	238	260	38 (7)*	9.90	6.88	7,150	31	5.75
	11th "		249	280	168	13.71	7.48	8,000	30	5.93
	22nd "		260	254	290	12.61	7.84	7,250	35	6.05
	1st "	2nd	289	260	38 (7)*	12.30	7.88	10,150	29	6.45
	14th "		303	272	136 (6)*	13.34	7.08	9,800	35	6.87
	28th "		317	261	290	15.56	8.12	10,400	35	7.09
120	1st "	1st	238	230	30 (5)*	9.90	6.80	9,000	32	6.45
	18th "		256	230	165	14.58	6.52	8,500	30	6.80
	22nd "		260	237	208	14.15	6.92	8,000	34	7.21
	1st "	2nd	284	262	34 (6)*	10.33	6.68	9,800	34	5.35
	13th "		297	257	170	12.30	8.44	11,350	35	6.39
	27th "		311	254	320	12.96	8.56	10,700	36	7.08
127	1st "	1st	273	225	31 (6)*	8.48	9.52	7,500	32	6.62
	14th "		287	212	112	11.68	7.24	8,840	29	5.63
	28th "		301	232	236	14.15	9.16	9,400	37	6.87
	1st "	2nd	329	223	27 (6)*	9.34	7.20	10,680	29	7.38
	15th "		344	258	133	9.72	7.96	10,700	30	7.09
	28th "		357	245	267 (5)*	11.93	8.28	12,700	36	7.38

* Numbers in parenthesis following weight records of litters indicate the number of young in the litter.

Summary

1. Age plays a determining rôle in the concentration of hemoglobin and erythrocytes in the blood of the albino rat during the period of growth. Sex plays a relatively insignificant role in the concentration of hemoglobin and erythrocytes.

2. The total leucocytes show such variation in individual animals during various stages of growth, that definite conclusions as to the influence of age and sex cannot be made.

3. Pregnancy, on our standard stock diet No. 1, produces an anemia in the albino rat which is more marked on day of delivery of young. With the advance of lactation the anemia disappears.

4. During advanced stages of pregnancy a decided drop in the concentration of serum proteins is apparent.

5. The concentration of hemoglobin and red corpuscles in the lactating rat is greatest near the weaning period of the young when the nurslings begin to partake of the maternal diet.

BIBLIOGRAPHY

1. Williamson and Ets, *Amer. Jour. Physiol.*, 1926, LXXVII, 480.
2. Williamson, *Arch. Inter. Med.*, 1916, XVIII, 505.
3. Sure, *Jour. Biol. Chem.*, 1926, LXIX, 65; Ibid., 1928, LXXVI, 695.
4. Hart, Steenbock, Elvehjem, and Waddell, *Jour. Biol. Chem.*, 1925, LXV, 67.
5. Newcomber, *Jour. Biol. Chem.*, 1919, XXXVII, 465.
6. Van Slyke and Neill, *Jour. Biol. Chem.*, 1924, LXI, 523.
7. Nicholas, *Amer. Jour. Physiol.*, 1928, LXXXIII, 499.
8. Sure, Kik, and Walker, unpublished data.
9. Happ, *Johns Hopkins Hospital Bulletin*, 1922, No. 33, 163.
10. Reiss, *Ergeb. d. inn. Med. u. Kinderh.*, 1913, X, 53.
    Marriott, *Physiol Rev.*, 1923, III, 275.
    Darrow and Buckman, *Amer. Jour. Dis. Child.*, 1928, XXXVI, 248.

THE EFFECT OF PURIFIED DIETS, AND THEIR MODIFICATIONS, ON GROWTH AND TESTICULAR DEGENERATION IN MALE RATS*

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INTRODUCTION

DURING the past fifteen years or so our knowledge in the realm of nutrition has been greatly enriched through the study and more complete understanding of the vitamin and mineral requirements of the animal organism. On the basis of these valuable findings it has been possible to compose diets in which the protein, carbohydrate and fat constituents are highly purified, adequately balanced, and of known chemical composition; and in which vitamin and mineral factors are supplied in amounts above the minimum requirements for growth and development. Such diets have usually proved adequate to produce splendid growth, health and vigor in the commonly used albino rat, yet these animals have almost invariably proved sterile after sufficient period of maintenance on such diets. The cause of such infertility has been the subject of extensive studies during the past seven or eight years, with the result that we are led to include another dietary factor in our group of accessory food factors—namely, the anti-sterility vitamin, or vitamin E, of Evans and Bishop ('22). The existence of this vitamin and its necessity for the normal reproductive function of both male and female rats have been demonstrated, in recent years, by the work of many investigators.

The female rat, probably because of its more rapid response to vitamin E deficiency, and the less serious nature of the disorder produced, has been the subject of much more extensive investigation of vitamin E

* This investigation was made in cooperation with Drs. Thomas B. Osborne and Lafayette B. Mendel, under whose direction the feeding of the experimental animals was conducted. The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

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sterility than has the opposite sex. However, the studies of Mattill, Carman and Clayton ('24), Evans ('25), Mason ('25; '26), Mattill and Clayton ('26), Evans and Burr ('27) and Mattill ('27) have added much to our knowledge of sterility in the male. The results of these investigators have shown that:—In male rats fed purified food diets, deficient in vitamin E, there is an initial period of fertility depending on the storage of vitamin E in the tissues. This is followed by a definite and progressive degeneration of the germinal epithelium of the testis. The extent of this degeneration may vary within rather wide limits in different tubules, or parts of tubules, in the same testis. Once the process is initiated, the germinal epithelium continues to degenerate and cannot be returned to normal, even though the animal is supplied with large amounts of vitamin E. However, the E factor may effect the retention of normal sperm-producing power in some tubules, or parts of tubules, in which the more remote physiological upset of the epithelium has not become evident histologically. The marked difference exhibited in the two sexes[1] resulting from lack of vitamin E, which has been adequately discussed by Evans and Burr ('27) in their splendid monograph, and by Mattill, Carman and Clayton ('24) and others, needs no further elaboration here. Whether we are dealing here with two separate factors specific for each sex, or with a single factor manifesting itself in a very different manner in the two sexes, is still a question. It was in the hope of adding a few more facts to our conception of the male sterility that the studies described in this paper were made.

Since our previous testicular studies (Mason, '26) were based chiefly on male rats fed a basal 18 per cent casein diet, it seemed advisable to investigate the sterility-producing effect of other "purified diets," especially a number which appeared to be unusually potent in their growth promoting properties. Also, the unusually rapid and extensive growth obtained in previous experiments, following the addition of fresh lettuce supplements, warranted further investigation. Furthermore, it seemed quite desirable to extend the histological studies to organs other than the testis in order to determine whether this lack of vitamin E in the diet produced a specific effect on the testis, or whether the accessory reproductive organs and other general body organs and tissues might also be

[1] Degeneration of the germinal epithelium of the testes in the male. Normal oestrous, ovulation, fertilization, migration and implantation in the female; but failure of the embryo to develop normally, resulting in death and resorption of the fetus on or about the thirteenth day of gestation. For a very complete account of the histopathology of gestation in "sterile" female rats, see Evans and Burr ('27 p. 9–23).

affected in some way. The results of these studies form the basis of this communication.

Materials and Methods

The total of over 200 male rats used in this study includes 45 animals on special experiment and over 160 animals, used by Osborne and Mendel in various "growth" experiments, which were turned over to the writer for autopsy and sterility studies.

At autopsy, the size, weight and macroscopical appearance of the testes were carefully noted. It has been found that the weight of the testis, as compared with Donaldson's ('24) figures for the normal testis weight (based on the body weight) is a fairly accurate indicator for the histological condition of the testis. From the study of the large number of animals, in which the above observations were checked with histological studies on the testes, we feel quite safe in stating that any testis less than 80 per cent normal by weight almost invariably shows degenerative changes. This is in close accord with the findings of Mattill and Clayton ('26). Degeneration is also indicated, macroscopically, by the hemorrhagic and translucent appearance of the tunica and the edematous condition of the testis. However, in more advanced stages the testis may appear very small, soft and flabby.

In addition to the above, smear slides from the testis and epididymis were prepared in a large number of cases and, after some experience and comparison with prepared sections of these organs, this method was found to be more accurate than the weight values in diagnosing the histological condition of the testis. However, in all but a few cases, the above observations were further sustained and corroborated by a study of histological sections of the testis and epididymis. A few cases were studied from a standpoint of sperm motility and a few matings were made, but these studies were not carried to any great extent. The other organs were examined grossly and, in a large number of cases, the following tissues (in addition to the testis and epididymis) were removed and fixed for histological study:—seminal vesicle, prostate, kidney, adrenal, spleen, pancreas, liver and thyroid.

Experiments

No extensive observations were made on the fertility of our male animals by means of mating experiments. Our attention was directed toward the ability of our various basal diets, and their modifications, to prevent or induce the characteristic testicular changes. Since the degenerative

process is apparently irreversible (Mason, '26; Evans and Burr, '27), it has been assumed that any diet which initiates these changes would eventually produce complete sterility. However, the fertility of the rat might continue for some time after the beginning of testicular degeneration, due to the great resistance of some of the tubules, or portions of tubules, to vitamin E lack. Previous studies (Mason, '26) showed that, in male rats reared from weaning on the basal 18 per cent casein diet, the testicular degeneration could be detected histologically after from 50 to 90 days, and no animals possessed perfectly normal testes after 100 days or more maintenance on the diet. Hence, in the present study, 100 days of feeding have been considered necessary before any conclusions as to the adequacy of the diet for fertility can be safely inferred.

The above mentioned 18 per cent casein diet has always proved excellent for growth in rats. A similar diet, in which the protein content was increased to 35 per cent casein with a corresponding reduction in the starch content, produced somewhat better growth. Hence, it seemed advisable to investigate the effect of such a régime on the testes, to assure ourselves whether the previously observed testicular changes on the 18 per cent casein diet were in any way concerned with an inadequate protein supply, or with an improper balance of the protein and carbohydrate components of the ration. Furthermore, because of the possible superiority of lactalbumin over casein for growth and development, as suggested by the observations of Osborne and Mendel ('17), this diet was modified by replacing the casein with lactalbumin and with edestin, at the 35 per cent level.

Previous observations (Mason, '26) on the prophylactic effect of fresh lettuce supplements to the 18 per cent casein diet also indicated the presence of some growth accelerating factor or factors in this substance, in addition to the fertility factor, vitamin E, as evidenced by a phenomenally rapid rate of growth following lettuce administration. No adequate explanation for this phenomenon was forthcoming. Although lettuce is known to contain considerable amounts of vitamin A, B and C, our basal diet was adequate in respect to these factors, and supplying them in excessive amounts had never produced any noticeable improvements in the growth rate. The 40 grams of fresh lettuce fed daily in these experiments, which would represent approximately 1.5 grams dried weight, could not account for the large daily increment in body weight over and above that of litter mate brothers fed the same basal diet but deprived of the lettuce. Therefore, coincident with the above dietary procedures,

a number of male rats were fed the basal synthetic diets supplemented with lettuce or alfalfa, in fresh and dried forms, and observations were made on the effect of these substances on growth and fertility.

The various types of diets used, as well as their general effects on growth and fertility, are summarized in Table I. It will be noted that, in every diet used, four of the components (viz; lard, butter fat, salts and yeast) remained constant. The protein component was either 18 per cent or 35 per cent casein, 35 per cent edestin, 35 per cent lactalbumin or 20 per cent or 30 per cent "meat residue."[2] The starch content was 54, 52, 42, or 37 per cent, depending upon the level at which the protein component was fed. In all cases these two components constituted 72 per cent of the entire diet. References to these diets in the text will be made by the designation 18 per cent casein, 35 per cent edestin, etc. The daily feeding of 10 drops of cod liver oil in some of the diets can be disregarded, as far as its effect on fertility is concerned, since it has been adequately shown by Evans and Burr ('27) and Sure ('27) that this oil is extremely poor or entirely lacking in vitamin E.

A. Changes in Quantity and Quality of Protein

Although the 35 per cent casein diet produced more rapid and better growth in male rats than did the 18 per cent casein diet, it was just as inadequate as the latter in the prevention of the testicular degeneration (diets 1 and 2, Table I). Histological examination of the testes of 19 rats fed the 18 per cent casein diet, and of 11 rats fed the 35 per cent casein diet (100 to 238 days), showed extreme degeneration in all cases, with stages 4 and 5 predominating.[3] Similar examination of other male rats, not included in Table I, reared on diets in which the protein content was increased to 60 per cent and 75 per cent casein, with a corresponding reduction in the starch content, gave the same result.

Replacing the casein of the diet with edestin or lactalbumin, at a 35 per cent level, or with meat residue, at a 20 per cent level, had no beneficial effect on the prevention of sterility (diets 2, 8, 10 and 25). This agrees with observations of Evans and Bishop ('23) on the lack of any superiority of lactalbumin over casein on reproduction in *female* rats reared on a

[2] Meat residue was prepared from bottom round steak, trimmed and ground at the market. This was suspended in liberal amounts of cold water, heated to boiling, filtered on cheese cloth and pressed in a hydraulic press. It was then ground fine, put into boiling water, boiled several minutes, drained, pressed, ground, dried in a hot water dryer and ground to a powder.

[3] These numbers in the text refer to the five stages in degeneration of the testes previously described by Mason ('26).

TABLE I

TO SHOW THE EFFECT OF THE BASAL DIETS, AND THEIR MODIFICATIONS, ON THE TESTES OF MALE RATS

Diet number	Dietary Regimen												Rats Used	Time on Experiment days		Rats % Normal by Weight		Testes % Normal by Weight		Remarks
	Casein	Edestin	Lactalbumin	Starch	Lard	Butter fat	Salts†	Yeast††	Cod Liver Oil	Alfalfa (dried)	Lettuce (fresh)	Lettuce (dried)								
	%	%	%	%	%	%	%	grams.	dr.	gm.	gm.	gm.		min.–max.	av.	min.–max.	av.	min.–max.	av.	
1	18			54	15	9	4	.2–.4					19	125–238	171	80–139	113%	43– 76	48%	Degen. adv. in all cases.
2	35			37	15	9	4	.2–.4					11	100–238	152	101–145	125%	38– 70	52%	Degen. adv. in all cases.
3	18			54	15	9	4	.2–.4	10				2	190–191	190	80–110	95%	49– 52	50%	Degen. adv. in all cases.
4	18			54	15	9	4	.2–.4		.4			8	100–179	152	108–156	135%	38– 70	50%	Degen. adv. in all cases.
5	18			54	15	9	4	.2–.4	10	.4			2	115–125	120	138 -162	150%	46–100	73%	One normal; other very degen.
6	35			37	15	9	4	.2–.4		.4			3	100–226	167	141–171	158%	58– 66	62%	Degen. adv. in all cases.
7	35			37	15	9	4	.2–.4	10	.4			4	170–230	187	139–174	156%	36–102	67%	One normal; lit. mate brother and two others showed adv. degen.
8		35		37	15	9	4	.2–.4	10				6	195–239	206	102–124	112%	31– 56	44%	Four cases very degen.; two mod.
9		35		37	15	9	4	.2–.4	10	.4			4	167–213	189	129–165	147%	38–110	60%	One case normal; lit. mate brother and two others showed adv. degen.
10			35	37	15	9	4	.2–.4	10				4	74–218	161	83- 153	110%	48– 87	60%	Three cases very degen. other rat on diet only 74 days—not suffi. time.

† Osborne, T. B., and Mendel, L. B., *Jour. Biol. Chem.*, 1919, XXXVII, 572.

††The yeast dosage was doubled in later stages of the feeding period on account of the greater requirement of vitamin B in older animals, as demonstrated by Osborne and Mendel ('25).

††The yeast dosage was doubled in later stages of the feeding period on account of its growth-promoting properties illustrated by Osborne and Mendel ('25).

TABLE I (Continued)

11	18			54	15	9	4	.2–.4			10		5	100–250	162	120–170	153%	92–121	109%	Testes normal.
12	18			54	15	9	4	.2–.4			20		3	100–250	192	145–182	165%	88–120	105%	Testes normal.
13	18			54	15	9	4	.2–.4			30		2	100–175	137	137–162	150%	90–	90%	Testes normal.
14	18			54	15	9	4	.2–.4			40		5	125–165	148	155–181	170%	100–111	106%	Testes normal.
15	35			37	15	9	4	.2–.4			5		3	291–293	292	126–162	139%	91–102	95%	Testes normal.
16	35			37	15	9	4	.2–.4			10		3	100–250	158	154–194	179%	93–100	97%	Testes normal.
17	35			37	15	9	4	.2–.4			20		3	100–304	218	156–166	162%	91–107	101%	Testes normal.
18	18			54	15	9	4	.2–.4				.34	2*	110–124	117	152–181	166%			Testes normal at op.;
													2**	165–238	201	142–154	148%			very degen. at autopsy.
																				One rat depr. of lettuce, after operation.
19	18			54	15	9	4	.2–.4				.68	1		250		180%		37%	Degen. adv. in all cases.
20	35			37	15	9	4	.2–.4				.34	3*	100–124	108	122–171	154%	91–100	96%	Testes normal at op.;
													3**	178–250	222	85–180	137%	39– 42	40%	very degen. at autopsy.
																				One rat depr. of lettuce after operation.
21	35			37	15	9	4	.2–.4				.68	1*		100		169%		100%	Testis normal at op.;
													1**		250		180%		42%	very degen. at autopsy.
22	35			37	15	9	4	.2–.4				1.6	2	224–238	231	130–170	150%	104–116	110%	Testes normal.
23		35		37	15	9	4	.2–.4				1.6	3	210–248	223	100–136	122%	73–113	97%	Two cases normal; other mod. degenerate.
24			35	37	15	9	4	.2–.4				1.6	1		262		128%		106%	Testes normal.
	Meat Residue																			
25	20			52	15	9	4	.2–.4					4	199–235	214	122–185	151%	45–63	52%	Four cases of adv. degen.
26	20			52	15	9	4	.2–.4	10	.4			2	237–242	239	141–162	146%	59–62	60%	Degen. adv. in all cases.
27	30			42	15	9	4	.2–.4	10	.4			4	137–165	149	115–185	155%	36– 55	46%	Degen. adv. in all cases.

* Data on rats up to operation and removal of left testis.

** Data on same rats from beginning of experiment to autopsy.

similar basal ration. Although the rate of growth in rats on the 35 per cent lactalbumin diets was excellent and well above the normal, it was somewhat inferior to that obtained on the 35 per cent casein and 35 per cent edestin diets. However, our data are too limited to suggest a possible superiority of these two proteins over lactalbumin, as far as growth itself is concerned. The excellency of the meat residue diet for growth has been demonstrated by Osborne and Mendel ('26—chart IV).

The six rats fed the 35 per cent edestin diet showed definite and marked testicular degeneration. Three rats fed the 35 per cent lactalbumin diet for periods of from 169 to 218 days showed complete degeneration of the testes. The testes of the other animal were normal, but the limited feeding period of only 74 days was not sufficient to induce the changes which undoubtedly would have occurred at a later date. The four rats fed the 20 per cent meat residue diet showed severe testicular degeneration.

It is clear, from the above, that a basal purified ration consisting of 37 per cent starch, 15 per cent lard, 9 per cent butter fat, 4 per cent salts and 0.2–0.4 grams of yeast, daily, the protein content of which is either casein, edestin or lactalbumin, fed at a 35 per cent level, or meat residue, fed at a 20 per cent level (starch 52 per cent), induced a rate of growth far superior to that of our normal stock rats, but proved just as inadequate as the 18 per cent casein basal ration in preventing the testicular changes characteristic of the "sterility disease."

B. Lettuce Supplements

The general results of these experiments are given in Table I, diets 11 to 24 inclusive. Reference to diets 11 to 17 shows that the daily supplement of 5 grams of fresh lettuce is just as efficacious as the 40 gram supplement in preventing testicular degeneration in male rats fed a sterility-producing diet. It is of interest that Evans and Burr ('27) have been successful in effecting "cures" in females by supplementing the basal diets with 2.5 grams of fresh lettuce, daily; but their employment of 1 gram, daily, was unsuccessful. It is also surprising to note that 5, 10, 20, 30 and 40 grams of fresh lettuce, fed daily, have resulted in approximately the same acceleration of growth. A typical example is shown in Chart I. Although the differences in rate and extent of growth in the four brothers given the lettuce supplements are not marked or constant, the general growth of these animals is much superior to that of the other brother (B 2357), fed the same basal diet minus the lettuce supplement. The testes of all the lettuce-fed rats were normal, both at operation (left

testes removed at 100 days) and at autopsy (150 days after operation), while those of B 2357 were in advanced degeneration in both instances.

The dried lettuce used was made into tablets, in two lots.[4] The tablets of lot 1 were used in diets 18, 19, 20 and 21. That the growth accelerating

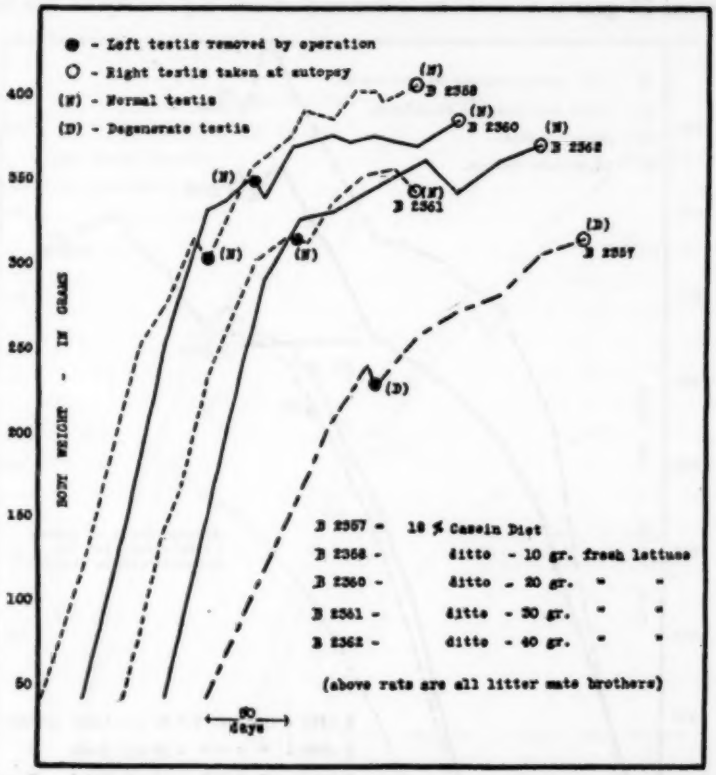

CHART 1.—Showing the marked stimulation of growth, in male rats, resulting when the basal 18% casein diet is supplemented with 10, 20, 30 and 40 grams of fresh green lettuce, daily.

[4] Lot 1 was produced from fresh lettuce bought at the market; lot 2, from lettuce gathered fresh from the field. The green leaves were dried for approximately 48 to 60 hours in a hot water circulation dryer at a temperature of 70°C. The dried lettuce was then ground to a fine powder and compressed into tablets, with the aid of a small amount of starch paste. In lot 1, each tablet represented the dried equivalent of 10 grams of fresh lettuce. Since the dried powder represented 3.37% of the original amount of fresh substance, each tablet then contained approximately 0.34 grams of desiccated lettuce. In lot 2, the dried powder represented 4.77% of its original weight and tablets were made up to contain .8 grams of the desiccated substance, corresponding to 16.77 grams of fresh lettuce.

properties of fresh lettuce are not appreciably diminished in the drying process, is clearly demonstrated in Charts 2 and 3. Although three of the four litter mate brothers shown in Chart 2 had an original weight of 47 grams, on the first day of experiment, and the remaining brother (B 2875) weighed 59 grams, there is no indication of any superiority of the fresh

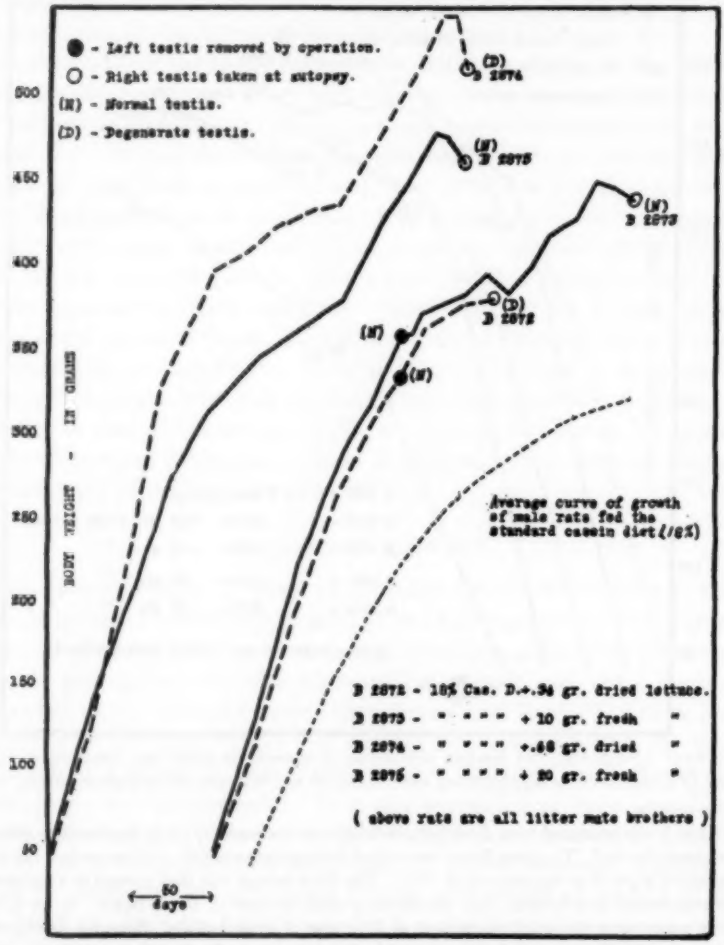
● - Left testis removed by operation.
○ - Right testis taken at autopsy.
(N) - Normal testis.
(D) - Degenerate testis.
BODY WEIGHT - IN GRAMS
500
450
400
350
300
250
200
150
100
50
(D) B 2874
(N) B 2875
(N) B 2873
(D) B 2872
(N)
(N)
Average curve of growth of male rats fed the standard casein diet (18%)
B 2871 - 18% Cas. D.+.34 gr. dried lettuce.
B 2873 - " " " + 10 gr. fresh "
B 2874 - " " " +.68 gr. dried "
B 2875 - " " " + 20 gr. fresh "
(above rats are all litter mate brothers)
50 days

CHART 2.—Showing a comparison between the growth-stimulating and sterility-preventing effects of fresh and dried lettuce, when fed as supplements to the basal 18% casein diet. The .34 and .68 gram supplements of dried lettuce represent the dried equivalent of 10 and 20 grams, respectively, of fresh lettuce.

lettuce, over corresponding amounts in dried form, as far as growth is concerned. The same conclusions are reached in the case of Chart 3. Rat B 2874 (Chart 2), given the .68 gram supplement of dried lettuce, shows a phenomenally rapid rate of growth, reaching a maximum body weight of 546 grams just seven days before autopsy. The superior growth

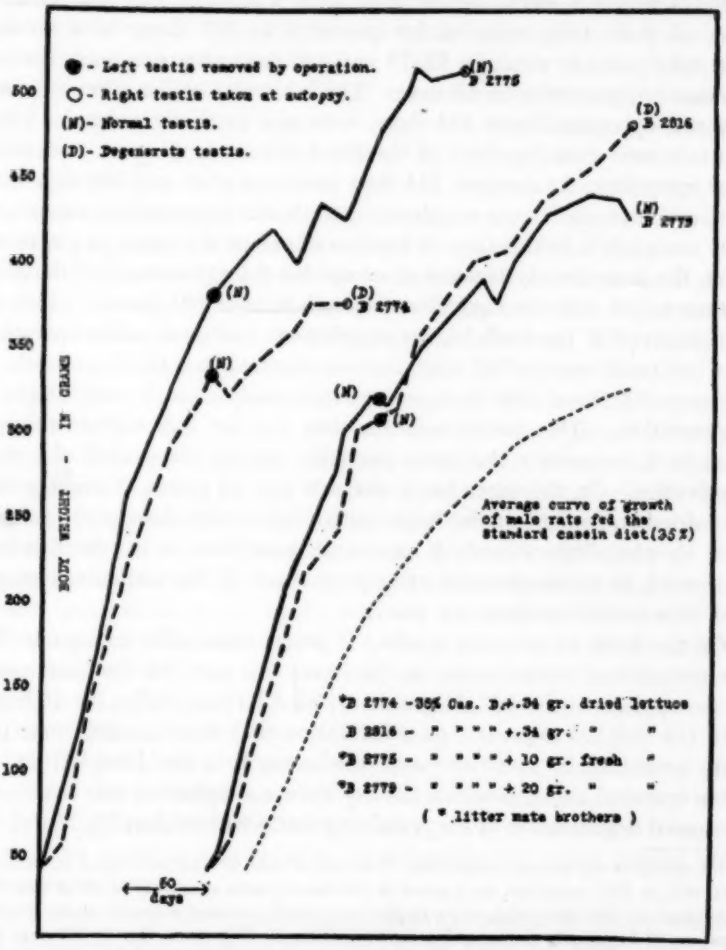

CHART 3.—Showing a comparison between the growth-stimulating and sterility-preventing effects of fresh and dried lettuce, when fed as supplements to the basal 35% casein diet. The .34 gram supplement of dried lettuce represents the dried equivalent of 10 grams of fresh lettuce.

of this rat is interesting but we have no satisfactory explanation, other than the individual variations in growth capacity observed in different rats.

Five rats of this group were given daily supplements of 1 dried lettuce tablet, representing 10 grams of fresh lettuce or .34 grams of dried lettuce, (diets 18 and 20) and had the left testis removed by operation 100, 100, 100, 124 and 124 days, respectively, after weaning. The left testes of three of these rats, removed by operation at 100 days, were normal. The right testes at autopsy, 55, 78 and 150 days after operation, showed advanced degeneration in all cases. The left testes of the other two rats, removed by operation at 124 days, were also perfectly normal. These two rats were then deprived of the dried lettuce supplement two weeks after operation. At autopsy, 114 days after operation and 100 days after deprivation of the lettuce supplement, testicular degeneration was practically complete in both cases. A brother of one of the above two rats was given the same dietary treatment, except for the replacement of the dried lettuce tablet with its equivalent of fresh lettuce (10 grams). This rat was deprived of the fresh lettuce supplement two weeks after operation. The left testis removed at operation was normal, but the right testis, at autopsy, 100 days after lettuce feeding ceased, showed early stages of degeneration. This would indicate that the rat had stored sufficient vitamin E to protect the testis partially, during the period of lettuce deprivation. On the other hand, the rats fed .34 grams of dried lettuce (the dried equivalent of the fresh lettuce fed to the above rat) probably used up what little vitamin E there may have been in the dried lettuce consumed, in an unsuccessful attempt to ward off the testicular changes, and were unable to effect any storage.

On the basis of previous results,[5] it seems reasonable to assume that the retention of normal testes in the above five rats, fed the basal casein diets supplemented with .34 grams of dried lettuce, daily, for 100, 100, 100, 124 and 124 days, is a good indication that these animals were partially protected by small amounts of vitamin E in the dried lettuce. In three operated cases, in which the dry lettuce supplement was continued, advanced degeneration of the remaining testes was evident 55, 78 and 150

[5] A survey of our records reveals that, in a total of over 45 male rats reared on either the basal 18% or 35% casein diet for a period of 100 days or more after weaning (10 of these were maintained for 100 days only), not a single one of these possessed a normal testis. Furthermore, out of 11 sexually mature males, maintained on the 18% casein diet for 100 days each, only one possessed a normal testis. We must infer then, since 98% of all male rats succumed to the "sterility disease" before the first 100 days of experiment, that the retention of a normal testicular condition beyond 100 days of feeding, especially in rats fed the basal ration from weaning, is of very rare occurrence.

days later. Hence, the vitamin E supplied in the .34 gram supplement was well below the minimum requirement of the rat for complete protection against sterility. This is supported by the fact that the daily addition of twice the above amount of dried lettuce (.68 grams = 20 grams of fresh lettuce) to the basal diet (diets 19 and 21) failed to prevent testicular changes in two male rats during a period of 250 days, although the left testis of one of these rats was normal at operation (100 days). The other animal was not operated upon. That this later degeneration of the remaining testes could not be due to trauma or any other factor resulting from operative procedure is almost certain, for the litter mate controls of this series, given fresh lettuce supplements, were similarly operated upon and all showed perfectly normal testes at autopsy. Furthermore, over 200 unilateral castrations performed during the past five years have never given any indication of operative injury to the remaining testis.

The dried lettuce of lot 2 was made up into tablets containing .8 grams of the dried substance (=16.7 grams of fresh lettuce). Unfortunately only one animal was given the daily .8 gram supplement. This rat showed no evidence of testicular degeneration, after 257 days on the diet, even though this represented less fresh lettuce than did the .68 gram supplement of lot 1, which failed to protect two male rats during the same 250 day period. However, since this .8 gram supplement was increased to 1.6 grams after 90 days of feeding, it is possible that the first supplement had a slight prophylatic effect which was sufficiently reinforced, by the additional increase at 90 days, to prevent any testicular changes. This assumption is further supported by the fact that supplementing the basal 35 per cent casein, 35 per cent edestin or 35 per cent lactalbumin diets with 1.6 grams of lot 2 (=33.5 grams of fresh lettuce) supplied sufficient vitamin E, in five out of six cases (cf. diets 22, 23 and 24), to prevent the testicular changes which otherwise would have intervened. The one case of degeneration observed was not very far advanced (chiefly stages 1 and 2). These six rats were maintained on the diets for periods of from 210 to 262 days, which is ample for an adequate test of the efficacy of the 1.6 gram supplement of dried lettuce. The growth of the rats of this group was of the same superior type as described for the other groups given lettuce supplements.

The above observations, although somewhat limited in extent, warrant the following deductions—(1) Additions of fresh or dried lettuce, as supplements to the basal rations, had approximately the same stimulating effect on growth in male rats regardless of whether they were fed in daily amounts of 5, 10, 20, 30 or 40 grams of fresh lettuce, or as the dried equiv-

alent of 10, 20 or 33.5 grams of the fresh leaf. (2) Although daily supplements of 5 grams of fresh lettuce were sufficient to prevent the testicular changes, which would otherwise occur in rats fed the basal diets, the dried equivalents of 10 or 20 grams of fresh substance (lot 1) failed to prevent these changes. However, there was some indication that the onset of these changes was delayed, to a slight extent, due to the presence of small amounts of vitamin E in the dried leaf. (3) Daily supplements of 1.6 grams of dried lettuce, the dried equivalent of 33.5 grams of fresh lettuce (lot 2), were efficacious in protecting five out of six males from the testicular changes, and greatly prolonged the onset of these changes in the other animal. (4) Since 5 grams of fresh lettuce were somewhat more effective than the dried equivalent of 33.5 grams, our dried lettuce was approximately one eighth as potent in vitamin E as was the fresh substance. (5) The growth-stimulating factor, in both fresh and dried lettuce, could not be shown to be related in anyway to the vitamin E factor, nor was it appreciably destroyed in the process of drying, as was the latter.

C. Alfalfa Supplements

In some of the "growth" experiments of Osborne and Mendel male rats were given 0.4 grams of dried alfalfa,[6] as daily supplements to the various basal diets. A number of these were autopsied and the testes examined histologically. Some of these rats had also been given 10 drops of cod liver oil daily but, as has been pointed out above (page 315), this substance is extremely poor or lacking in the E factor and hence could not appreciably alter the sterility results of this group. The growth curve and general physical condition of the rats were excellent in every instance. The data for these animals is summarized in Table I, diets 4, 5, 6, 7, 9, 26 and 27.

Out of the 27 cases studied, all but three rats showed advanced stages of testicular degeneration, after periods of maintenance on the diet varying from 100 to 242 days. Three rats had perfectly normal testes after 125, 175 and 177 day periods of maintenance on diets 5, 7 and 9, respectively. It is also interesting to note that the litter mate brothers of two of these three rats, given the same dietary treatment, showed advanced testicular degeneration. The above three instances of unusually long retention of normal testes must be due either to an unusually high resistance of these particular animals to lack of the E factor, or to an increased intake of

[6] The alfalfa was cut when about 8 to 10 inches high and growing rapidly. It was brought immediately to the laboratory and put in the hot water dryer on wire bottom trays. After about 24 hours drying (at 70°C) the leaves were shaken off, and most of the large stalks removed, leaving a product consisting almost entirely of leaves and tender stems. This was ground to a powder and made into tablets.

vitamin E. Since the alfalfa was fed daily in separate tablets, and no uneaten tablets were observed in the cages, the intake of dried alfalfa was constant and could be in no way influenced by the level of food intake. The only other way in which these three alfalfa-fed rats could have secured, more vitamin E than the other twenty-four was through an increased food intake and the consequent increase of butter fat, and other dietary components, which might contain small amounts of vitamin E. That increased food intake is not an adequate explanation is indicated by a comparison of the two pairs of brothers mentioned above. One pair (B 2244 and B 2246) was fed the 35 per cent casein basal diet, plus 0.4 grams of dried alfalfa and 10 drops of cod liver oil, daily, for 175 days after weaning. B 2244 (original wt. 49 grams), with a slightly better growth rate throughout the experiment, and an average weekly food intake of 86.6 grams, showed moderate testicular degeneration. B 2246 (original wt. 43 grams) with an average weekly intake of 81.5 grams, had perfectly normal testes. The other pair, with very similar growth records, showed corresponding differences in average weekly food intake—90.2 grams for the rat with very degenerate testes; 80.3 grams for the other.

A careful consideration of the above data leads one to the conclusion that, barring animals which for some unknown reason have an unusually high resistance to lack of vitamin E, the daily addition of 0.4 grams of *dried* alfalfa, to our sterility-producing diets, has little or no prophylatic effect. As to the effect of *fresh* alfalfa additions, we have little evidence to present. One group of four males, fed the 35 per cent casein diet plus 5–10 grams of fresh alfalfa, daily, was terminated, for other reasons, at 70 days. The testes were normal, but this was too early in the experiment to indicate the possible prophylatic effect of the fresh substance.

D. Additional Observations

In addition to the above studies, a large number of male rats fed various other diets were examined for their testicular condition. Over 40 male rats, fed diets of which yellow corn meal (58%, 70% and 79%) and skimmed milk powder (30%, 29% and 20%) formed the chief components, to which were added various amounts of salt, iron, iodine, liver (dried), yeast and cod liver oil, exhibited perfectly normal testes, even after long periods of feeding on the diets. The success of these diets was apparently due to their high content of yellow corn meal, which Evans and Burr ('27) have shown to contain considerable quantities of vitamin E. Other rats, fed the basal 35% casein diet supplemented with 0.4 and 0.6 grams of dried beef liver, daily, have invariably shown testicular degeneration. This confirms the observations of Evans and Burr ('27) on the low content of vitamin E in liver tissue.

Autopsies made on several hundred sterile male rats, representing a large variety of dietary régimes, have revealed nothing of significance—exclusive of the reproductive tract. Lung infections were occasionally met with, but this condition was no more frequent in sterile rats than in normal fertile rats. Likewise, microscopical studies on the adrenals, thyroids, pancreas, liver, spleen and lymph glands of these rats were negative. However, the epididymis, and in some cases the prostate and seminal vesicle, have shown interesting changes which appear to be a natural consequent of the degeneration of the testis, and not a primary resultant of lack of vitamin E. Studies on these tissues are still in progress and will form the subject of a later communication.

Furthermore, a few blood studies warrant brief mention here. Hart, Steenbock, Elvehjem and Waddell ('25) have suggested a possible relationship between vitamin E and iron assimilation, and the relation of these two factors to hematin formation in the body. More recent work of Simmonds, Becker and McCollum ('27) also indicates a similar relationship.[7] We have made a number of comparisons between the total erythrocyte count and total hemoglobin content of the blood in male rats fed diets deficient, and diets abundant, in vitamin E. Although repeated estimations were made on the same animals, at various intervals, the erythrocyte and the hemoglobin content were normal in all cases studied.

Discussion

In a consideration of vitamin E, and its relation to dietary sterility, two phases of the problem have assumed paramount interest for us—viz., the relation between vitamin E, sterility and growth; and the specificity of the vitamin in the two sexes.

The acceleration of growth rate produced after the addition of various quantities of lettuce supplementing the basal "purified food" diets, which in themselves are capable of producing excellent growth, naturally leads one to question the possibility of the presence of some other growth-stimulating factor, aside from the E vitamin, in such supplements. This growth-accelerating factor manifests itself in stimulated and superior growth of rats when approximately 0.4 grams of dried lettuce or of dried alfalfa are added as daily supplements to our basal diets, even though these supplements are too deficient in their vitamin E content to prevent testicular degeneration. Furthermore, supplementing the same basal diets with increased amounts of fresh or dried lettuce, more than sufficient to maintain fertility, has failed to produce growth superior to that obtained

[7] These investigators have since abandoned this theory as untenable (*This Journal*, 1928, I, 39.), thus strengthening our negative evidence.

when the small 0.4 gram supplements of dried material were fed. It is also of interest to note that these supplements not only stimulate the actual rate of growth but also make it possible for animals to attain a greater body weight than is possible on the basal diets alone. Likewise, removal of the supplement from the diet soon results in lowered rate of growth and decreased growth capacity. Hence, we appear to be dealing here with some factor capable of accelerating the metabolism and assimilation of some component or components of the diet, or with some factor capable of releasing some such metabolic accelerator previously inhibited.

The data so far secured have given us no definite clue as to the identity of this growth-accelerating factor, introduced in our lettuce and alfalfa supplements. However, three possibilities present themselves—the factor may be, (1) a greater supply of the growth-promoting vitamins A and B, (2) vitamin E itself, or (3) some unknown growth-promoting factor.

That vitamin A is not concerned seems assured, for cod liver oil supplied in supplements of 10 to 20 drops daily, together with the A vitamin supplied in the butter fat at the 15% level in the diet, has never evoked such growth stimulation in male rats fed the basal purified diets. Regarding the part played by the water-soluble B vitamin, we can only say that such a growth acceleration has not been produced by the addition of greater quantities of vitamin B, in the form of yeast, to the basal diets. The above observations on vitamins A and B are amply confirmed and extended in the growth studies of Palmer and Kennedy ('27). These investigators have observed an almost universal growth failure in rats fed a highly purified basal diet, not unlike that used in our experiments. Their casein was probably more rigidly purified than that used in our experiments and their supply of vitamin B was from a different source (alcoholic extract of ether extracted wheat embryo). They found that when one or all of the known vitamins were added to the diets, in greatly excessive amounts, there was no improvement in the growth rate which could be attributed to increased amounts of vitamins A, B, C, D, E or bios. However, some instances of improved growth following excessive vitamin administration appeared to be due to factors present in the vitamin-carrying substances or their extracts. Similar growth-promoting factors were found to be present in dried muscle meat and in commercial casein, the latter showing an apparent "seasonal variation in growth-promoting value not associated with recognized vitamins." It seems assured that vitamins A, B, C and D play little or no part in this growth acceleration.

In regard to the possible implication of vitamin E, it may be of some interest to note the observations of several investigators. Evans and Burr

('27) state that "in attempted cures the administration of wheat germ oil almost always led to superior growth, as it did in cured animals. We are, unfortunately, not in the position to state whether or not this insignificant accretion to the energy needs of the body (six drops of wheat germ oil daily) would have a similar benefit were vitamine E omitted." In a later paper ('27b) these investigators observe that their highly purified casein—sucrose—salt mixture diet, supplemented with 700 milligrams of yeast and 2 drops of cod liver oil, daily, was deficient in some substance other than vitamins A, B, D or E, as indicated by a slow growth rate plateauing at about 100 to 125 grams, and by abnormalities in the ovulation cycle. And replacement of the yeast supplements with 750 milligrams of fresh wheat germ, daily, gave a somewhat more inferior growth in both males and females. This deficiency was readily corrected when the diet was further supplemented with 10 grams of fresh lettuce or with 0.5 grams of fresh beef liver. They also state ('27c) that additions of dried lettuce, and of the ether extract of dried lettuce, did not noticeably improve the growth deficiency of the diet, indicating that "a considerable destruction of the active substance present in fresh lettuce leaf had resulted from the drying process." The differences in the diets used, and their failure to state the amount of dried lettuce supplied in their supplements, make it difficult to compare their findings with the more successful results obtained by us with dried lettuce. Possibly their more rigidly purified diet required more of this unexplained growth factor, than did the diets used in our experiments. Mattill and Clayton ('26) state that "a temporary but marked acceleration of growth took place in animals of both sexes when wheat germ or wheat germ oil was added to the basal ration, whether fertility was subsequently evoked or not." The basal diet of these investigators was practically identical with our 18% casein diet. Furthermore, Palmer and Kennedy ('27) make the following comment—"Lack of vitamin E cannot be judged as the cause of the growth abnormalities in spite of the evidence that unsaponifiable residues from wheat germ oil stimulated growth slightly in our experiments. Inasmuch as vitamin E plays an important rôle in the development of the fetus, it should not be surprising that it is to be classified among the growth-promoting factors." Unfortunately, our experimental series described in this paper did not include any studies on wheat germ oil supplements. However, more recent studies on the effect of supplementing A-deficient and D-deficient diets with daily additions of 10 and 20 drops of wheat germ oil have shown little or no growth stimulation following the administration of this substance. The same was true for guinea pigs fed a C-deficient diet.

That vitamin E, *per se*, is not responsible for this growth stimulation

seems quite evident when we consider the following facts—(1) It has been confirmed by various investigators (Evans and Burr, '27; Mattill and Clayton, '26; Palmer and Kennedy, '27) that wheat germ oil, a potent source of the anti-sterility factor, produces only a slight or temporary growth stimulation. (2) Remarkable growth stimulation was observed in our male rats fed the small 0.34 and 0.68 gram supplements of dried lettuce, and 0.4 gram supplements of dried alfalfa, even though these additions failed to supply sufficient vitamin E to protect the testes against the degenerative changes. And administration of large amounts of fresh lettuce, up to 40 grams daily, introducing more than eight times the amount of the anti-sterility E factor necessary to prevent testicular degeneration, has not produced growth superior to that attained with the dried lettuce supplements. (3) Osborne and Mendel ('26) have shown a similar growth acceleration when the basal casein diet is supplemented with 0.4 grams of dried liver, daily. Evans and Burr ('27b) have made similar observations, using 0.5 grams of fresh beef liver, and have also shown ('27) that the vitamin E content of liver is very small; 5 grams of beef liver, daily, being necessary to effect "cures" in female rats.

Yet, we cannot positively exclude the possibility that vitamin E may consist of two factors—a growth-promoting and a sterility-preventing factor—not unlike the dual nature recently described for vitamin B by various investigators. If such be the case we must further assume that, in the case of male rats fed a basal "purified" food diet inadequate for fertility, small amounts of the E vitamin may be sufficient for the growth-stimulating function, whereas much larger amounts are necessary to supply sufficient of the anti-sterility factor for normal testicular function. Such a supposition might explain the remarkable growth observed in our male rats fed the small 0.34 and 0.68 gram supplements of dried lettuce, and the 0.4 gram supplements of dried alfalfa, even though these additions failed to prevent testicular degeneration. However, the recently published studies of Evans ('28a) present additional evidence that vitamin E is in no way concerned in the growth stimulation under discussion in this communication. Evans ('28a) finds that supplementing his basal diets with small amounts of wheat germ oil (6 drops, daily), or with small amounts of the more concentrated E-containing fractions of this oil, produces a marked improvement in growth and general physical condition of his rats, which he attributes to the beneficial effect of vitamin E. That this growth stimulation observed by Evans in his animals is of a somewhat different nature from that observed in our experiments, and described in this paper, seems quite evident. Evans states that; "The improvement in growth due to vitamin E characterizes only the last phase of growth in

these animals, the phase intervening after the attainment of maturity, which is usually viewed as a plateau in the growth curve though all animals show at this time a slow ascent. The improvement becomes increasingly evident after the eighth month of life, and is very marked after one year." On the other hand, the growth acceleration observed in our experiments appears very soon after lettuce feeding is begun, but has never been followed beyond the eighth month of life. It would appear that we are dealing here with two different phenomena, each attributable to entirely separate causative agents. What, then, is the cause of the growth acceleration observed in our experimental animals?

We still have one alternative which offers a much more hopeful explanation. While our experimental results have indicated the presence and operation of some unknown factor, in the production of this growth stimulation, they did not seem to justify such a postulation until further verified. However, the interesting growth studies of Palmer and Kennedy ('27) and Kennedy and Palmer ('28; '28a) have so corroborated our findings that we are led to assume that the growth-stimulating factor concerned in our experiments is similar to, if not identical with, the unknown growth factor described by these investigators, and shown to be unrelated in any way to any of the known vitamins A, B, C, D, or E or bios. Further investigation is necessary to solve the identity of this factor. All we can say, at present, is that commercial casein, dried beef muscle (Palmer and Kennedy, '27), dry starch-free yeast (Kennedy and Palmer, '28), rat feces (Kennedy and Palmer, '28a), dried or fresh liver (Osborne and Mendel, '26; Evans and Burr, '27b), fresh lettuce, dried lettuce and dried alfalfa appear to contain some unknown and unrecognized factor, or factors, which greatly stimulates growth in rats fed "purified food" diets, containing adequate amounts of the other known vitamins.

As to the identity and nature of this factor, or factors, we can say very little. Kennedy and Palmer ('28; '28a) have shown that the factor in dry starch free yeast is only slightly extracted from the yeast by acidulated water or alcohol, and withstands autoclaving for 2 1/2 hours at 15 pounds pressure; whereas the factor in rat feces is alcohol-soluble, but not ether soluble, and is stable to heating for 1/2 hour at 15 pounds pressure but showed deterioration after 2 hours heating. These investigators state that the evidence at hand does not point to the identity of these two factors. Yet, it seems possible that these physical differences, above described, may be due to a different physical or chemical association of the same growth-promoting factor in the two substances—dry starch-free yeast and rat feces. They are also unable to identify their growth factors with the growth factor described by Smith and Hendrick ('26) as being

present in brewers' yeast and as a necessary supplement to diets in which the sole source of protein is casein or oat kernel. However, Kennedy and Palmer ('28) do admit a close similarity between their factor, present in yeast, and that of the latter investigators, as well as with the P–P factor of Goldberger and Tanner ('25).

From the investigations reviewed in this paper, and from many other nutritional studies of the past few years, it would appear that we are still far from a true conception of the complete growth requirements of the normal animal organism, and of the various factors concerned in the process of growth.

Evans and Burr ('27) have been successful in obtaining "cures" in females by supplementing their basal diets with .25 and .5 grams of dried lettuce, and by supplementing the same diet, deprived of its fat content, with .6 grams of dried alfalfa, daily. Our inability in preventing the testicular changes in males with .34 and .68 grams of dried lettuce and with .4 grams of dried alfalfa, and our partial success with 1.6 grams of dried lettuce, indicate either a higher minimum requirement for fertility in the male, or else a greater destruction of vitamin E in our process of drying. It is quite probable that male rats, due to their larger size and greater metabolic demands, do have a slightly higher vitamin E requirement than do female rats, fed the same basal diet. But this difference in requirement can not be very marked, for 5 gram supplements of fresh lettuce, fed daily, have sufficed to protect our male rats; and Evans and Burr ('27) have found that the minimum requirement to effect "cures" in female rats, fed a similar basal diet, lies somewhere between 1 and 2.5 grams. Unfortunately, we have no data on the efficacy of the 2.5 gram, or smaller, supplement on the male rat. Our results have also indicated a considerable loss of vitamin E in the drying of fresh lettuce. Evans and Burr ('27) give no data regarding this. Their lettuce was dried more rapidly, and at a somewhat higher temperature, than was that used in our experiments. Whether such differences in the drying process would noticeably influence the vitamin E content of the dried substance is impossible to state at present. The success of these investigators in curing female rats with daily supplements of .6 grams of dried alfalfa, and our failure with .4 grams, may be due to the fact that their alfalfa was fed as a supplement to a fat-free basal ration, thereby decreasing the amount of vitamin E necessary for fertility.[8]

[8]It has been shown by Evans and Bishop ('23), Sure ('27a) and other investigators, that milk fat, although low in vitamin E, does contain sufficient amounts to confer fertility, when fed at high levels in purified food diets. Mattill, Carman and Clayton ('24); Mattill ('27a); and Evans and Burr ('27a) have also demonstrated that the presence of lard and certain other fats

That the germ cells of the male rat require a definite amount of vitamin E for their complete metamorphosis would seem assured—providing adequate amounts of the vitamins A, B, and D are supplied. Yet it seems difficult to conceive of the same factor having no relation to germ cell maturation, ovulation or implantation in the female; but performing here a definite function in relation to placentation and embryonic development, as has been so admirably set forth by Evans and Burr ('27) in their recent monograph. However, the observations of various investigators of dietary sterility have failed to give any indication that the same factor—vitamin E— is not responsible and necessary for the maintenance of fertility in both the male and female rat. In fact, the results of various investigators indicate that the dietary cause of sterility, the vitamin E requirement for fertility, the storage of vitamin E in the tissues of the body and the "initial fertility" observed on rats reared from weaning on inadequate diets, are practically identical in both sexes, regardless of the marked differences in the histo-pathological manifestations of the "sterility disease."

Thus we are led to assume that vitamin E is a specific requirement of the germinal epithelium of the testis, and that lack of this factor has no demonstrated effect on any other organ or tissue of the body (page 326). The specificity of this vitamin for the male sex gland is further demonstrated in the recent work of Mattill ('27). In this very interesting paper, Mattill has shown that male rats, even when exhibiting extreme signs of vitamin B deficiency, usually possess normal testes provided adequate amounts of vitamin E are supplied. However, such animals may show marked infertility, which Mattill states, "is believed to be due to the loss of tone and vigor and the consequent lowered sex expression coincident with the lowered level of metabolism. This is delayed but not prevented by vitamin E." These findings have also been corroborated in a recent paper by Evans ('28). This work represents an important step towards ascertaining the specificity of vitamin E for the male sex gland. Still further investigation along this line is necessary to determine whether the sterility previously described by many investigators, and attributed

greatly reduces the efficiency of vitamin E-containing components of the diet. However, in the purified diets used for the studies described in this paper, we have retained the same proportions of butter fat (9%) and lard (15%) which were present in the basal 18% casein diet, used in our previous studies on testicular degeneration. This was done because of the greater ease in comparing our observations on growth and fertility, following various modifications of this basal diet, with those previously obtained. Hence, we must admit the presence of small amounts of vitamin E already present in our "purified" diets, but this factor is relatively constant and, at the same time, quite inadequate to confer fertility. The salt and yeast components were also present in the same amounts as in our basal 18% casein diet.

to a large variety of dietary deficiencies, is due to absence or insufficiency of the vitamin E factor alone.

We have been investigating the effect of wheat germ oil administration on animals fed various vitamin-deficient diets, and in animals subjected to partial and total inanition. The results so far secured indicate that the specificity of vitamin E for the male gonad is not so definite and clear cut as the results of previous experiments would indicate. A complete report on this work will be made in the near future.

Summary

The testicular degeneration, previously described as occurring after feeding male rats on a basal "purified" diet (18% casein, 54% starch, 15% lard, 9% butterfat, 4% salts and .2–.4 grams of yeast, daily), has been found to occur equally as rapidly in animals fed similar dietaries in which the protein constituent was casein, edestin or lactalbumin, at a 35% level, or meat residue, at a 20% or 30% level.

Daily supplements of 5, 10, 20, 30 or 40 grams of fresh green lettuce or 1.6 grams of dried lettuce (the dried equivalent of 33.6 grams of fresh lettuce), supplied sufficient vitamin E to prevent testicular degeneration in male rats reared on the "purified" basal diets. Daily supplements of .34 and .68 grams of dried lettuce, and of .4 grams of dried alfalfa, were inadequate.

Fresh lettuce was found, in these experiments, to be approximately eight times as rich in vitamin E as its dried equivalent.

Both the fresh and dried supplements contained some factor capable of inducing an unusually rapid rate of growth, as well as an increased growth capacity, in male rats. The possible relation of this factor to vitamin E and to other growth factors is discussed.

The erythrocyte and hemoglobin content of the blood of sterile male rats, fed diets deficient in vitamin E, were normal.

Histological examination of the adrenals, thyroids, pancreas, liver, spleen and lymph glands of sterile male rats has failed to reveal histopathological changes.

Bibliography

Donaldson, H. H. 1924—The Rat: Data and Reference Tables. Second edition. *Amer. Anatomical Memoir* No. 6.

Evans, H. M. 1925—Invariable occurrence of male sterility with dietaries lacking fat soluble vitamine E. *Proc. Nat. Acad. Sci.*, 1925, XI, 373.

—— 1928—The effect of inadequate vitamin B upon sexual physiology in the male. *This Journal*, 1928, I, 1.

—— 1928a—Relation of vitamin E to growth and vigor. *Ibid.*, 1928, I, 23.

Evans, H. M., and Bishop, K. S. 1922—On the existence of a hitherto unrecognized dietary factor essential for reproduction. *Science*, 1922, LVI, 650.

—— 1923—The production of sterility with nutritional régimes adequate for growth and its cure with other foodstuffs. *Jour. Metab. Res.*, 1923, III, 233.

EVANS, H. M., and BURR, G. O. 1927—The antisterility vitamine fat soluble E. Memoirs Univ. of California, *Vol.* VIII.

—— 1927a—Vitamin E I. The ineffectiveness of curative dosage when mixed with diets containing high proportions of certain fats. *Jour. Amer. Med. Assoc.*, 1927, LXXXVIII, 1462.

—— 1927b—A new dietary deficiency with highly purified diets. *Proc. Soc. Exp. Biol. and Med.*, 1927, XXIV, 740.

—— 1926c—New dietary deficiency with highly purified diets. II. Supplementary requirement of diet of pure casein, sucrose and salt. *Proc. Soc. Exp. Biol. and Med.*, 1927, XXV, 41.

GOLDBERGER, J. and TANNER W. F. 1925—A study of the pellagra-preventive action of dried beans, casein, dried milk and brewers' yeast, with a consideration of the essential preventive factors involved. *Pub. Health. Rep.*, 1925, XL, 54.

HART, E. B., STEENBOCK, H., ELVEHJEM, C. A., and WADDELL, J. 1925—Iron in nutrition. I. Nutritional anemia on whole milk diets and the utilization of inorganic iron in hemoglobin building. *Jour. Biol. Chem.*, 1925, LXV, 67.

KENNEDY, C. and PALMER, L. S. 1928—The fundamental food requirements for the growth of the rat. III. Yeast and yeast fractions as a supplement to synthetic rations. *Jour. Biol. Chem.*, 1928, LXXVI, 591.

—— 1928a—The fundamental food requirements for growth of the rat. IV. Coprophagy as a factor in the nutrition of the rat. *Ibid.*, 1928, LXXVI, 607.

MASON, K. E. 1925—A histological study of sterility in the albino rat due to a dietary deficiency. *Proc. Nat. Acad. Sci.*, 1925, XI, 377.

—— 1926—Testicular degeneration in albino rats fed a purified food ration. *Jour. Exp. Zool.*, 1926, XLV, 159.

MATTILL, H. A. 1927—The relation of vitamins B and E to fertility in the male rat. *Amer. Jour. Physiol.*, 1927, LXXIX, 305.

—— 1927a—The oxidative destruction of vitamins A and E, and the protective action of certain vegetable oils. *Jour. Amer. Med. Assoc.*, 1927, LXXXIX, 1505.

MATTILL, H. A., CARMAN, J. S., and CLAYTON, M. M. 1924—The nutritive properties of milk. III. The effectiveness of the X substance in preventing sterility in rats on milk rations high in fat. *Jour. Biol. Chem.*, 1924, LXI, 729.

MATTILL, H. A., and CLAYTON, M. M. 1926—Vitamin E and reproduction on synthetic and milk diets. *Ibid.*, 1926, LXVIII, 665.

OSBORNE, T. B., and MENDEL, L. B. 1917—The relative value of certain proteins and protein concentrates as supplements to corn gluten. *Ibid.*, 1917, XXIX, 69.

—— 1925—The rôle of vitamine B in relation to the size of growing rats. *Ibid.*, 1925, LXIII, 233.

—— 1926—The relation of the rate of growth to diet. I. *Ibid.*, 1926, LXIX, 661.

PALMER, L. S., and KENNEDY, C. 1927—The fundamental food requirements for the growth of the rat. II. The effect of variations in the proportion and quality of recognized nutrients. *Ibid.*, 1927, LXXV, 619.

SIMMONDS, N., BECKER, J. E., and MCCOLLUM, E. V. 1927—The relation of vitamin E to iron assimilation. *Jour. Amer. Med. Assoc.*, 1927, LXXXVIII, 1047.

SMITH, M. I., and HENDRICK, E. G. 1926—Some nutrition experiments with brewers' yeast with especial reference to its value in supplementing certain deficiences in experimental rations. *Pub. Health. Rep.*, 1926, XLI, 201.

SURE, B. 1927—Dietary requirements for reproduction, IX. Cod liver oil versus wheat oil as sources of vitamin E. *Jour. Biol. Chem.*, 1927, LXXIV, 45.

—— 1927a—Dietary requirements for reproduction. XI. The potency of butter fat in vitamin E. *Ibid.*, 1927, LXXIV, 71.

RELATION OF SOIL FERTILITY TO VITAMIN A CONTENT OF LEAF LETTUCE*

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THE vitamin A content of plant tissue has been shown to vary with its degree of greenness or chlorophyll development.[1] Does it vary with plant vigor, or, in other words, with the conditions of soil nutrition which determine plant development with respect to size and robustness? The results reported herein bear upon this question.

Selected seed of the Grand Rapids Forcing variety of leaf lettuce was sown in successive lots in clean bench sand. Eight or ten days later, when the seedlings were about 2.5 inches high, they were transferred singly to six-inch flower pots, each pot containing five pounds of uniform medium coarse sandy loam soil that was very low in fertility. These pots were divided into three groups; each group treated differently as to the soil, and all kept under identical environal conditions during the growth of the plants. In one group, "S," the soil was left in its natural state; in another, "M," 25 grams of pulverized sheep manure plus 1 gram of a mixture of C.P. KNO_3, $CaH_4(PO_4)$ and KCl, in the proportions of 1:2:1 grams were added to the soil of each pot; while in a third group, "F," each pot received a full unit of this inorganic mixture (4 grams total) but no organic manure. By these soil treatments plants of three distinct sizes and degrees of vigor, as shown in Figure 1, were secured.

Table I gives the chemical composition of the plants. Analyses were made by the Experiment Station Chemist using the following method: nitrogen, Kjeldahl; potassium, Kramer's micro-method; calcium, McCrudden; iron, A.O.A.C. colorimetric; manganese, Willard and Greathouse's colorimetric; phosphorus, Brigg's modification of Bell-Doisy's colorimetric; aluminum, aurine tricarboxylic method.

The vitamin A content of the lettuce was determined by the rat growth method. Young rats, 28 to 30 days old from the laboratory colony, were depleted of their store of vitamin A by feeding an irradiated vitamin

* Published as Paper No. 5 (n.s.) from Michigan State College Agricultural Experiment Station.

[1] Dye, M., Medlock, O. C., and Crist, J. W., *Jour. Biol. Chem.*, 1927, LXXIV, 95.

FIG. 1.—Representative plants from the three soil treatments. Left to right F, M, S.

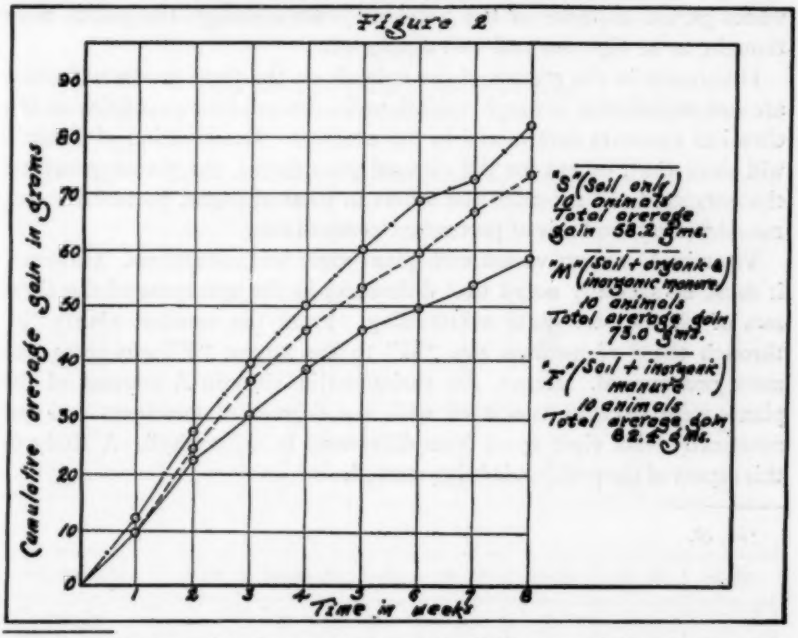
TABLE I

PARTIAL CHEMICAL ANALYSES OF LETTUCE PLANTS*

Plant	Water per cent	Nitrogen per cent	Potassium per cent	Calcium per cent	Iron per cent	Manganese per cent	Phosphorus per cent	Aluminum per cent
S	92.9	3.72	6.50	1.88	0.105	0.006	0.348	0.058
M	94.2	3.10	5.84	1.14	0.016	0.004	0.444	0.021
F	92.8	2.44	5.70	0.98	0.016	0.010	0.450	0.022

* Water in fresh material, nitrogen and minerals on oven dry basis.

A-free ration.[2] The general procedure was described in a former paper from this laboratory.[3] The feeding of lettuce was started either when xerophthalmia was present or when definite losses in weight occurred. Two-tenths of a gram of lettuce grown on each type of soil was fed daily per animal. The results are given graphically in Figure 2.



[2] The basal ration used in these experiments was irradiated cornstarch 78 per cent, purified casein 18 per cent and salt mixture (McCollum's No. 185) 4 per cent. Each animal received daily 0.5 grams of dried Fleischmann yeast.

[3] *loc. cit.*

The cumulative growth curves of Figure 2 are based upon the average gains in weight made by groups of ten animals fed each type of lettuce. Each group made excellent gains in weight for the first two weeks, but the one receiving lettuce "S" grew at a slower rate through the ensuing weeks. The animals fed the lettuce raised on soil treated only with inorganic fertilizer "F" grew at the most rapid rate and made the greatest gain during the experimental period of eight weeks. While the curve of the group given lettuce "M" follows closely the former, it is at all times below it and increasingly so towards the end of the experiment. The total gains for the three groups in the experimental period of eight weeks are as follows: Lettuce F—82.4 gms., M—73.0 gms., and S—58.2 gms.

In comparing these results with those obtained two years[4] ago it is interesting to find that the average gain of the rats receiving 0.2 grams of lettuce "F" daily was 18.9 grams (30 per cent) greater than that made by the former group that was fed 0.3 grams of lettuce grown on ordinary soil. The group given lettuce "S" made almost as great a gain in the eight weeks period as those of the previous years although the plants were thought to be vigorous and well developed.

Differences in the growth of the animals on the three grades of lettuce are not explainable through variations in the relative quantities of the chemical elements determined in the analyses. Examination of Table I will show that, except for the element phosphorus, the plants grown on the untreated soil exceeded the others in total nitrogen, potassium, etc., considered on the basis of percentage composition.

Vitamin A content varied with plant vigor and robustness. However, it must be specially noted that differences in the greenness of the three sets of plants were quite outstanding. From the smallest plants "S" through those of medium size "M" to the largest "F" greenness was more pronounced. Hence, the variations in vitamin A content of the plants were likewise associated with the degrees of greenness and not necessarily with vigor apart from differences in chlorophyll. A study of this aspect of the problem is being started.

[4] *loc. cit.*

OBESITY AS A PRECURSOR OF DIABETES*

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THAT obesity is one of the most common and most significant precursors of diabetes is generally accepted. So far as I am able to determine, the relationship between obesity and diabetes, as reported in the literature, has been investigated in urban districts. Data in The Mayo Clinic have been derived from the examination of patients coming, largely, from rural districts.

I studied 673 case histories in which the height and weight of each patient had been recorded at the time of the examination of the patient at the clinic. In each instance, the maximal weight and the age at that weight were known. Two groups of figures therefore were obtained; one group consisted of values obtained before the onset of diabetes; the other,

TABLE I

DATA SECURED BEFORE ONSET OF DIABETES

Per cent overweight	Age						
	1–10	11–20	21–30	31–40	41–50	51–60	61–70
91–100					8	5	
81– 90			2		7	6	1
71– 80			1	4	3	4	3
61– 70			1	2	11	18	5
51– 60		1	3	3	35	26	6
41– 50			5	7	20	29	12
31– 40		1	6	15	31	31	21
21– 30	1	5	8	16	27	34	23
11– 20	1	5	14	13	26	34	12
1– 10	2	9	11	9	16	15	5
Total	4	21	51	69	184	202	88
Per cent underweight	**Age**						
	1–10	11–20	21–30	31–40	41–50	51–60	61–70
11– 20		2	5	5	3		1
1– 10	2	6	10	9	8	3	
Total	2	8	15	14	11	3	1

* From the Division of Medicine, The Mayo Clinic, Rochester, Minnesota.

of similar values obtained after the onset of the disease. In each group, the percentage of overweight or underweight was determined by comparing the weight, and the age at that weight, with actuarial tables (Tables I and II). I therefore had for comparison the percentage of overweight and of underweight of a group of patients before and after diabetes developed.

From an analysis of these figures, it appears that 91 per cent of the patients were overweight and that 82.9 per cent were more than 10 per cent overweight before their diabetes began. When this broad and important generalization is examined in detail, two more facts emerge. In the first place, it appears (Table I) that as age increases to the fifth decade, the

TABLE II

DATA SECURED AFTER ONSET OF DIABETES

Per cent overweight	Age						
	1–10	11–20	21–30	31–40	41–50	51–60	61–70
91–100					1	1	
71– 80			1		1	1	
61– 70				1	3	2	
51– 60			1	2	3	2	1
41– 50			4	6	9	7	
31– 40			1	3	15	13	6
21– 30		1	3	4	24	27	6
11– 20		1	4	7	29	33	12
1– 10	3	5	7	13	35	37	27
Total	3	7	21	36	120	123	52

Per cent underweight	Age						
	1–10	11–20	21–30	31–40	41– 50	51–60	61–70
51– 60			1				
31– 40		1	3	5	6	1	3
21– 30		6	7	11	10	18	3
11– 20	2	7	16	10	27	22	13
1– 10	1	10	16	18	26	80	18
Total	3	24	43	44	69	91	37

candidates for diabetes increase in weight; after the age of fifty, the percentage of overweight is slightly lower. In the second place, it appears that obesity may be an important factor in the development of diabetes in young persons, because more than 30 per cent of persons less than twenty years of age were more than 10 per cent overweight before their diabetes began.

The figures in Table II were obtained after the patients had had diabetes for varying lengths of time. It will be seen that when diabetes became established about as many patients were underweight as were overweight. In some instances the figures for patients less than twenty years of age were approximate, because of the possible increase in height in persons of this age group. A slight error also occurs in the comparison of data concerning patients with those concerning questionably normal persons for which the actuarial tables are made.

TABLE III

TABULAR SUMMARY OF RESULTS

		Age						
		1–10	11–20	21–30	31–40	41–50	51–60	61–70
Before Diabetes	*Overweight*							
	Mean (per cent)	13.0	16.5	27.5	30.6	40.0	38–7	34–8
	Standard deviation (per cent)	8.3	12.7	21.4	18.4	23.3	21.9	17.5
	Probable error of mean (per cent)	2.8	1.9	2.0	1.5	1.1	1.1	1.3
	Underweight							
	Mean (per cent)	5.5	8.0	8.0	9.1	8.2	5.5	15.5
	Standard deviation (per cent)		4.4	4.7	4.8	4.5		
	Probable error of mean (per cent)		1.0	0.8	0.9	0.9		
After Diabetes	*Overweight*							
	Mean (per cent)	5.5	9.8	25.0	23.3	22.7	21.1	14.5
	Standard deviation (per cent)		7.3	19.9	18.1	17.3	16.2	11.8
	Probable error of mean (per cent)		1.9	2.9	2.1	1.1	1.0	1.1
	Underweight							
	Mean (per cent)	12.2	14.7	15.7	16.2	14.9	12.2	13.1
	Standard deviation (per cent)	4.7	9.1	10.9	10.5	9.3	8.3	9.1
	Probable error of mean (per cent)	1.8	2.0	1.1	1.2	0.8	0.6	1.0

A survey, preliminary to this problem, showed little relevant difference between the sexes. Males and females therefore were grouped together. No discrimination was used in the selection of these cases. The most

obese patients were not chosen for this study. These records were obtained from the data concerning 673 patients who had diabetes, and who presented themselves in the clinic because of diabetes, or because of something else, or both.

For convenience, obesity may be classified as preventable and unpreventable. Many persons who are overweight present a combination of the two classes. Preventable obesity is the type acquired because of faulty habits of living. A sedentary life may contribute to the acquisition of too much fat. Persistent overeating is frequently responsible for obesity. Wealth may encourage a sedentary life and may contribute, in part, to the cause of obesity. The idea that reduction can be easily accomplished may be a cause of obesity in certain persons; this idea may be the result of the widespread campaigns advertising the efficacy of reducing pills, corsets, massage and so forth. The unpreventable causes of obesity may be age, sex, heredity, previous diseases and various disturbances of the endocrine glands including those which take place at the menopause.

I am unable to determine the percentage of cases in this series in which obesity was preventable. It is my impression, however, that such cases form considerably more than half of the entire group. Of 1000 patients with diabetes whose records were examined, 579 gave a history of occasional or persistent overeating. One hundred and nine said that they ate sweets to excess; sixty-one ate inordinately of meat; only four admitted excessive use of alcohol, and one drank great quantities of grape juice. Joslin believes that it is the excessive amount of total food rather than the amount of carbohydrate which does the harm. He points out that the Japanese as a race consume a large amount of carbohydrate but that, in spite of this, they are not so prone to diabetes as are other races. Furthermore, he shows that when the disease does occur in a Japanese, it is usually mild. A recent report on the average diet of various countries indicates that in the United States consumption of food per capita is greater than it is in any other country. This may account, in part, for the alarming increase of diabetes in this country.

Education should help in preventing diabetes, since obesity seems to be such an important precursor of the disease. When people realize that a gain of weight is as significant a sign as acute pain is a significant symptom, timely steps may be taken to reduce the incidence of diabetes.

THE HEAT PRODUCTION OF THE ALBINO RAT
I. TECHNIQUE, ACTIVITY CONTROL, AND THE INFLUENCE OF FASTING

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THE albino rat, owing to its ease of handling and rapidity of breeding, has become the most frequently used animal in those laboratories dealing with problems of nutrition. At the inception of our research[1] in 1921 there was a paucity of information with regard to the metabolism and especially the energy exchange of this animal. As the result of a suggestion of Professor H. H. Donaldson of the Wistar Institute of Anatomy and Biology, whose continued interest in this research we gratefully acknowledge, it was proposed to make a survey of the metabolism of the albino rat with special reference to the influence of age, sex, environmental temperature, and fasting. A second feature of the survey was to accumulate data for use in a comparison of the basal metabolism of the rat with the basal metabolism of humans and other animals being studied by the Nutrition Laboratory, such as large ruminants, wild birds, and snakes. Since this research began a number of papers on the rat have appeared, a few of which anticipate some of our own findings, which have been delayed in publication. To make this report unified, but with no intention of claiming priority, we will record our data as needed in the discussion, likewise citing those publications that bear upon the same points.

The rats studied belonged to two well-established colonies at Columbia University, that of Professor H. C. Sherman of the Department of Chemistry and that of the Department of Nutrition at Teachers College. Both of these originated from animals obtained from the colony of Professor L. B. Mendel at Yale University, and by their high fertility, low death rate, and generally excellent health have proved to be normal and suc-

[1] In the carrying out of this long series of experiments we were assisted by Miss Margaret G. Barwis and Miss Mary Shimanovsky, and we are most appreciative of their intelligent and faithful cooperation.

cessful colonies. All of the rats studied were fed the same stock diet consisting of one-third whole milk powder, two-thirds whole wheat (ground) and common salt amounting to 2 per cent of the weight of the wheat. The protein content of this stock diet furnished 16 per cent of the total calories, which corresponds well with the present-day standards for protein in an "adequate" diet for humans. The rats studied were of stock that had lived on this diet exclusively for twenty or more generations.

The life of the albino rat is strikingly at variance with that of the wild Norway rat. The latter experiences extreme changes in environmental temperature and is excessively active, whereas the albino rat has, under modern conditions of handling, become a highly inbred laboratory animal, living under fairly uniform conditions of temperature and in most laboratories without special arrangement for exercise. Since this general life environment obtains in most rat colonies, we have studied this type of rat rather than that accustomed to muscular exercise in the highly ingenious cages of Slonaker.[3]

Studies of the Respiratory Exchange of Rats by Other Investigators

For studying the gaseous metabolism of an animal as small as the albino rat only the chamber method can be used. The earliest form of apparatus for small animals was that described in 1892 by Haldane,[2] whose technique, with some modifications, has been employed by numerous investigators since then in determining the gaseous metabolism of rats. Pembrey[4] was the first to report experiments on rats, using a somewhat modified form of the Haldane apparatus, with which he measured only the carbon-dioxide production. Later Pembrey and Spriggs[5] used this same apparatus for determining both the carbon-dioxide production and the oxygen consumption of normal rats. Harris[6] fitted up a respiration chamber in a manner somewhat similar to the arrangement in the Haldane-Pembrey apparatus. Campbell[7] also used the Haldane-Pembrey method with which to determine the carbon-dioxide production, but the oxygen consumption he measured by means of a "circulation method" involving the attachment of a Douglas bag to the respiration chamber,

[3] Slonaker, J. R., *Anat. Record*, 1908, II, 116.
[2] Haldane, J., *Jour. Physiol.*, 1892, XIII, 419.
[4] Pembrey, M. S., *Jour. Physiol.*, 1895, XVIII, 363.
[5] Pembrey, M. S., and E. I. Spriggs, *Jour. Physiol.*, 1904, XXXI, 320.
[6] Harris, D. T., *Proc. Roy. Soc. London*, Ser. B, 1925, XCVIII, 171.
[7] Campbell, J. A., *Proc. Roy. Soc. London*, Ser. B, 1926, XCIX, 451.

with gas analyses before and after one-hour periods of air circulation between the bag and the chamber. Harris and Campbell were both interested chiefly in the biologic action of light.

The Haldane apparatus was likewise used by Cramer,[8] who, although studying chiefly rats having tumors, had some control rats. Danoff,[9] of the Asher school in Berne, made use of the Haldane apparatus in 1919 in studying rats, and since that time the apparatus has been regularly employed by Asher and Abelin[10] and their students in all of their researches, both the carbon-dioxide production and the oxygen consumption being determined. Demaria Massey[11] has also measured the metabolism of rats by the Haldane method, with the Danoff technique. By a combination of the Haldane type of respiration apparatus with the Regnault-Reiset type, a respiration chamber for small animals was constructed by Fridericia,[12] in which the oxygen consumption of the animal could be determined in two independent ways, both directly and indirectly. The apparatus was thus self controlled.

The most ingenious attempts of Arnoldi (whose apparatus for rats is described by Asada[13] and briefly by Händel[14]) to register graphically the changes in weight of the various parts of the Haldane system have unfortunately not been sufficiently successful to justify the regular use of his technique. Verzár,[15] in 1924, reported results of experiments on white rats carried out in his laboratory by Árvay with the Haldane method. Subsequently Árvay[16] adopted the Nutrition Laboratory technique,[17] as described on pages 348 to 353 of this article.

Among the more recent investigators to employ modifications of the Haldane method have been Reiss and Weiss,[18] Mitchell and Carman,[19]

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[8] Cramer, W., *Third Sci. Report Imp. Cancer Research Fund*, 1908, 427; Cramer, W., and R. M'Call, *Quart. Jour. Expt. Physiol.*, 1917, XI, 59.

[9] Danoff, N., *Biochem. Zeitschr.*, 1919, XCIII, 44.

[10] See their numerous contributions, chiefly in the Biochemische Zeitschrift.

[11] Demaria Massey, C., *Compt. Rend.*, 1927, XCVII, 405.

[12] Fridericia, L. S., *Biochem. Zeitschr.*, 1913, LIV, 92.

[13] Asada, K., *Biochem. Zeitschr.*, 1923, CXLIII, 390.

[14] Händel, M., *Biochem. Zeitschr.*, 1924, CXLVI, 420.

[15] Verzár, F., *Verhandl. d. deutsch. Naturforscherversammlung in Innsbruck*, 1924. (Cited by A. Zih, *Pflüger's Arch. f.d.ges. Physiol.*, 1926, CCXIV, 449.)

[16] Árvay, A., *Pflüger's Arch. f.d.ges. Physiol.*, 1926, CCXIV, 421.

[17] Benedict, F. G., *Abderhalden's Handb. d. biolog. Arbeitsmethoden*, 1924, Abt. IV, Teil 10, 537 et seq.

[18] Reiss, M., and R. Weiss, *Klin. Wochenschr.*, Jhrg. IV, 1925, 2207.

[19] Mitchell, H. H., and G. G. Carman, *Amer. Jour. Physiol.*, 1926, LXXVI, 385.

Terroine and his associates,[20] and Lee.[21] The latter laid special emphasis upon the metabolism during the oestrous cycle.

A gravimetric method for the measurement of carbon dioxide only is reported briefly by Gulick.[22]

The Pettenkofer principle was applied by Pott[23] in 1875 in his study of animals of different species, among which he includes rats. Falloise,[24] in 1901, studied the influence of environmental temperature upon the metabolism of various warm-blooded animals, and included one experiment with a rat made in an open-circuit apparatus. An open-circuit method, permitting the determination of the carbon-dioxide production by absorption in soda-lime, was used with rats by J. J. R. Macleod[25] in 1907. Ten years later Prince[26] described an open-circuit respiration chamber for rats and other small animals, in which a current of air was drawn through the chamber and a sample of the outgoing air analyzed for oxygen and carbon dioxide. Krzywanek,[27] in 1923, reported results on rats obtained with his modification of the Zuntz-Geppert apparatus, and in 1926 Arnoldi[28] published experiments carried out by the use of Krzywanek's technique. An open-circuit method was likewise employed by Artundo,[29] who took the precaution of controlling his technique by comparison with the Haldane method and found that his results were on the average about 5 per cent above the results obtained by the Haldane method. Grafe[30] and his colleagues have described a "universal respiration apparatus" with different sizes of chambers adaptable for humans or small animals, such as the rat. The apparatus embodies the Jaquet principle of continuous aliquoting with an open circuit on the Pettenkofer-Zuntz basis. The outgoing air is analyzed by the Carpenter gas-analysis apparatus, both oxygen and carbon dioxide being determined.

The closed-circuit apparatus, in which either the oxygen consumption alone is measured or (by weighing the carbon dioxide absorbers) likewise the carbon-dioxide production, has been employed in a number of labora-

[20] Terroine, E. F., S. Trautman, and J. Schneider, *Ann. de Physiol.*, 1926, II, 468; Terroine, E. F., and S. Trautman, *Ann. de Physiol.*, 1927, III, 422.

[21] Lee, M. O., *Amer. Jour. Physiol.*, 1927, LXXXI, 492; ibid., 1928, LXXXVI, 694.

[22] Gulick, A., *Amer. Jour. Physiol.*, 1925, LXXII, 204.

[23] Pott, *Landw. Versuchsstationen*, 1875, XVIII, 81.

[24] Falloise, A., *Arch. de. Biol.*, 1901, XVII, 761.

[25] Macleod, J. J. R., *Amer. Jour. Physiol.*, 1907, XVIII, 1.

[26] Prince, A. L., *Jour. Biol. Chem.*, 1917, XXXII, 333.

[27] Krzywanek, Fr. W., *Biochem. Zeitschr.*, 1923, CXXXV, 506.

[28] Arnoldi, W., *Zeitschr. f.d. ges. exp. Med.*, 1926, LII, 249.

[29] Artundo, A., *Compt. Rend.*, 1927, XCVII, 407.

[30] Grafe, E., Fr. Strieck, and J. Otto-Martiensen, *Zeitschr. f.d.ges. expt. Med.*, 1927, LIV, 613.

tories. Thus the Kestner apparatus, which embodies the Regnault-Reiset and the Atwater-Benedict principles, was used by Cohnheim and v. Dungern[31] and later by Leichtentritt[32] for studying day-old rats, and by Groebbels[33] for determining the influence of low environmental temperatures. The Regnault-Reiset principle was also adopted by Giaja[34] and his associates. Giaja's investigations have had particular reference to the influence of environmental temperature, since his respiration chamber can be immersed in water at any desired temperature. With the Rohde type of closed-circuit apparatus[35] Hildebrandt[36] and Nishiura[37] made measurements of both respiratory gases in investigating the effects of thyroxin, arsenic, iron, phosphorus, and the like. The Hildebrandt type of apparatus was also used by Gabbe[38] and Seel[39] to study the effect of drugs and vitamin-deficient diets, by de Veer[40] to study the influence of ovarian extract, and by Pulewka[41] to study the action of acetylene gas.

Aszódi's[42] closed-circuit apparatus for mice was modified by Goto[43] for the study of rats. Mattill[44] employed the Chauveau and Kaufmann closed-chamber method. With the apparatus of Bijlsma,[45] also having a closed circuit, the oxygen consumption alone was measured by graphic registration of the fall in height of the spirometer bell, and with the closed-circuit apparatus of Foster and Sundstroem[46] and of Wesson[47]

[31] Cohnheim, O., *Zeitschr. f. physiol. Chem.*, 1910, LXIX, 89; Cohnheim, O., and E. v. Dungern, Eppendorf-Festschr., 1914, 33.

[32] Leichtentritt, B., *Zeitschr. f. Biol.*, 1919, LXIX, 545.

[33] Groebbels, F., *Pflüger's Arch. f.d.ges. Physiol.*, 1925, CCVIII, 661; Abderhalden's Handb. d. biol. Arbeitsmethoden, 1926, Abt. IV, Teil 10, 861.

[34] Giaja, J., *Ann. de Physiol.*, 1925, I, 596.

[35] Rohde, E., *Zeitschr. f. physiol. Chem.*, 1910, LXVIII, 181.

[36] Hildebrandt, F., *Arch. f. exp. Path. u. Pharm.*, 1921, XC, 330; *ibid.*, 1922, XCII, 68; *ibid.*, 1923, XCVI, 292.

[37] Nishiura, S., *Arch. f. exp. Path. u. Pharm.*, 1924, CI, 153; Hildebrandt, F., and S. Nishiura, *ibid.*, 1924, CI, 161; Nishiura, S., *ibid.*, 1924, CII, 320.

[38] Gabbe, E., *Klin. Wochenschr.*, 1924, Jhg. III, 612; *Zeitschr. f. d. ges. exp. Med.*, 1926, LI, 391 and 447.

[39] Seel, H., *Arch. f. exp. Pathol. u. Pharm.*, 1928, CXXVII, 212; *loc. cit.*, 1928, CXXVIII, 102.

[40] de Veer, A., *Zeitschr. f.d.ges. expt. Med.*, 1925, XLIV, 240.

[41] Pulewka, P., *Arch. f. exp. Path. u. Pharm.*, 1926, CXII, 82; *ibid.*, 1927, CXXIII, 267.

[42] Aszódi, Z., *Biochem. Zeitschr.*, 1921, CXIII, 70.

[43] Goto, K., *Biochem. Zeitschr.*, 1923, CXXXV, 107.

[44] Mattill, H. A., *Jour. Biol. Chem.*, 1923, LV, 717.

[45] Bijlsma, U. G., *Verslagen en Mededeelingen betreffende de Volksgezondheid*, August, 1926.

[46] Foster, G. L., and E. S. Sundstroem, *Jour. Biol. Chem.*, 1926, LXIX, 565; Foster, G. L., and P. E. Smith, *Jour. Amer. Med. Assoc.*, 1926, LXXXVII, 2151; Smith, P. E., C. F. Greenwood, and G. L. Foster, *Amer. Jour. Pathol.*, 1927, III, 669.

[47] Wesson, L. G., *Jour. Biol. Chem.*, 1927, LXXIII, 499.

both the carbon-dioxide production and the oxygen consumption were measured.

Direct calorimetric determinations with animals as small as rats have been undertaken by A. V. and A. M. Hill[48] and by Wagner.[49] A modified form of the Tangl[50] electric compensation calorimeter was used for rats by Hári[51] and by Aszódi.[52]

The results of many of these investigators deal with rats under conditions other than normal. In every research here cited, however, at least a few determinations were made on normal rats serving as controls, and for this reason the techniques employed have been cited and the normal data have frequently been taken into consideration in the interpretation of our own data.

Technique

Respiration apparatus employed in this research

The apparatus used in our research consisted of a modification of the closed-circuit respiration apparatus which has been employed in various forms in the Nutrition Laboratory. Both the oxygen consumption and the carbon-dioxide production were measured, and hence the respiratory quotient could be determined. This latter determination is of itself extremely advantageous, since when respiratory quotients approximating 0.71 are obtained during fasting, the accuracy of the measurements is strengthened. A short preliminary description of this apparatus, with diagram, was given in 1924.[53] The general scheme of the apparatus is shown in Figure 1.

The animal is placed in a small, open-mesh, metallic cage, which rests upon a metallic base having a water trough serving as a water seal. A standard glass baking dish (for bread) of heat-resistant glass is inverted over the cage into the water trough. This makes the interior of the apparatus light so that the animal is visible, and when in the light the rat

[48] Hill, A. V., *Jour. Physiol.*, 1912, xliv, i; Hill, A. V. and A. M. Hill, *Jour. Physiol.*, 1913, xlvi, 81.

[49] Wagner, R., *Zeitschr. f. Biol.*, 1925, lxxxii, 114; Abderhalden's Handb. d. biol. Arbeitsmethoden, 1926, Abt. IV, Teil 10, 845.

[50] Tangl, F., *Biochem. Zeitschr.*, 1913, liii, 21.

[51] Hári, P., *Biochem. Zeitschr.*, 1913, liii, 116; Abderhalden's Handb. d. biol. Arbeitsmethoden, 1925, Abt. IV, Teil 10, 711; Hári, P., *Biochem. Zeitschr.*, 1924, cli, 449.

[52] Aszódi, Z., *Biochem. Zeitschr.*, 1924, cli, 472.

[53] Benedict, F. G., Abderhalden's Handbuch d. biolog. Arbeitsmethoden, 1924, Abt. IV, Teil 10, fig. 221, p. 538. Also described by E. Krauss, Lehrbuch der Stoffwechselmethodik, I. Teil, Methodik des Energie- und Stoffwechsels, Leipzig, 1928, 270.

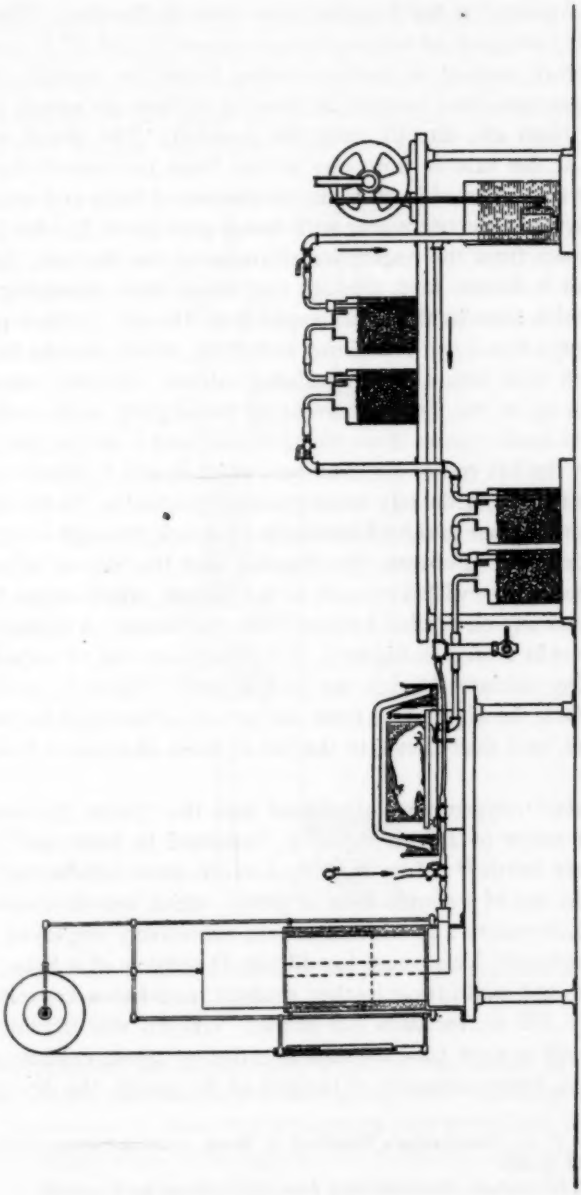

FIG. 1.—*A closed-circuit respiration chamber for rats.*

(a nocturnal animal) is much quieter than when in the dark. Most of the experiments were made at temperatures between 25° and 28° C., controlled by a moderate amount of electric heating below the metallic chamber. Cold temperatures were secured by blowing outdoor air mixed, as necessary, with room air, directly over the chamber. The closed system is ventilated at the rate of from one to two liters per minute by a small rotary blower, immersed in oil to insure absence of leaks and actuated by a chain drive and electric motor with worm gear above it. Air is sucked by this blower from the respiration chamber at the bottom, right-hand opening and is drawn, first, through two metal cans containing calcium chloride, which absorbs the water vapor from the air. It then passes up through a pipe into a can containing soda-lime, which absorbs the carbon dioxide, and next into a can containing calcium chloride, where water vapor given up to the dry air current by the slightly moist soda-lime is removed. It finally passes down to the blower and is discharged from the blower into the left end of the chamber, which is also in direct communication with a small, delicately counterpoised spirometer. In the pipe leading from the chamber to the spirometer is a petcock, through which oxygen may be admitted. Between the chamber and the blower is inserted a vertical piece of pipe with a petcock at the bottom, which serves to collect and withdraw any oil carried forward from the blower. A system of stopcocks, shown in section in Figure 1, and a duplicate set of soda-lime can and following calcium-chloride can (not shown in Figure 1), make it possible to switch the air current from one set of carbon-dioxide absorbents to the other, and thus alternate the use of these absorbents from period to period.

At the start oxygen was introduced into the system by means of a small Bohr meter of 250 cc. capacity, immersed in water and provided with a safety bottle.[54] More recently a much more satisfactory method has been the use of a simple form of pump, which was developed at the Nutrition Laboratory and which has been extensively employed in many of its researches.[55] This pump (see Figure 2) consists of a brass cylinder with piston and well-fitting leather washers, and has a capacity of approximately 370 cc. for each full stroke. Oxygen was drawn into the pump through a glass tube containing a drying agent, calcium chloride. By turning a 3-way stopcock at the end of the pump, the dry gas could

[54] Benedict, F. G., Abderhalden's Handbuch d. biolog. Arbeitsmethoden, 1924, Abt. IV, Teil 10, fig. 197, p. 465.

[55] Benedict, F. G., *Boston Med. and Surg. Jour.*, 1927, CXCVII, fig. 5, p. 1165.

then be discharged directly into the respiration apparatus. A rubber bag, such as a football bladder, served as an oxygen container. This was filled with oxygen from a cylinder of the compressed gas and was attached to a 3-way stopcock at the upper end of the calcium-chloride tube, so that relatively pure oxygen was drawn into the pump. To insure complete

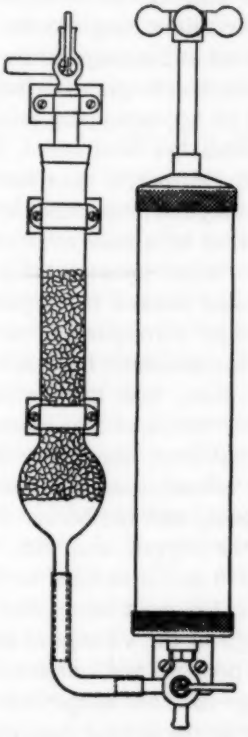

FIG. 2.—Pump of known, constant volume, for metering oxygen introduced into the closed circuit of the respiration apparatus.

A calcium-chloride tube at the left dries the oxygen.

atmospheric pressure, the upper 3-way stopcock was turned a moment to the air just before the contents of the pump were discharged into the system. This method of measuring the oxygen introduced into the apparatus has been found most advantageous and extremely accurate. In fact, any error entering into the observations has been not in the measurement

of the discharge from the pump but in the calculation of the volume of air residual in the respiration chamber at the end of each period. After this research was completed, it was found equally advantageous, on the basis of other studies in the Nutrition Laboratory, to measure the oxygen saturated with water vapor rather than dry. Hence the calcium chloride tube attached to the pump may be done away with and the oxygen in the basket-ball or football bladder can be saturated by having a small amount of water inside the rubber bag. Under these conditions one is dealing with oxygen saturated at the temperature of the pump.

The carbon-dioxide production is quantitatively determined by weighing the soda-lime can and its accompanying calcium-chloride can upon a 3-kg. Sauter balance,[46] which has been found, with full load, to have a sensitivity of 5 milligrams, i.e., better than that claimed by the manufacturer. The oxygen consumption is measured by noting the fall in height of the spirometer bell as read on a scale over which a pointer, attached to a counterweight, passes. When a pumpful of pure oxygen is introduced into the system at the point marked O_2 (Figure 1), the spirometer bell immediately rises to a height corresponding to the volume introduced. By reading the height of the spirometer bell before the pumpful of oxygen is introduced, noting the time, then introducing the oxygen, and terminating the period of observation at that moment when the spirometer bell has attained its original level, one knows the time required for the absorption of the definite volume of oxygen represented by the pumpful. The temperature of the pump and the barometric pressure are noted at the time of introducing the oxygen, and with these data the apparent volume of the pumpful (370 cc.) is reduced to 0° C. (dry) and 760 mm. Obviously a certain correction must be applied to this reduced volume to take care of any change in the volume of air residual in the system caused by changes in its pressure and temperature during the period of measurement. The average chamber temperature is read on a thermometer attached to the cover of the animal cage by a bit of adhesive tape, and the temperature of the absorbing vessels is indicated by a thermometer hung near them in the room.

From these two values for the carbon-dioxide production and the oxygen consumption one readily obtains the respiratory quotient, which has been found with rats 16 hours after food to be almost invariably 0.72 or

[46] Made by August Sauter, Ebingen, Württemberg, Germany. Balance No. 511 (old No. 8 II b).

0.73.[57] Respiratory quotients higher than 0.73 or 0.74 under these conditions are instantly indicative of errors in the apparatus. On the other hand, experiments made immediately after the animals have eaten should and do show respiratory quotients much higher.

The length of the experimental period was determined by the fact that it was necessary to collect in the soda-lime can at least one gram of carbon dioxide, in order not to have the error of weighing (i.e., ± 5 mgm.) play too great a role. Ordinarily a period of approximately 2 1/2 hours was required for a 200-gram rat, and it was usually possible to obtain two or three successive periods in an experiment on any one day.

Graphic Record of Activity

Because of the well-known influence upon human metabolism of even the moderate muscular movements of crossing the legs or of shivering, it was felt necessary at the inception of this research on rat metabolism to secure graphic evidence of the degree of repose of each animal during each experiment, in order to determine the effect of activity in the case of the rat. Hence every experiment made in this research was accompanied by a graphic record of activity. The Nutrition Laboratory has long stressed the importance of relying not only upon ocular observation, with periodic records, but upon some more or less automatic device which will register the slightest changes in muscular repose. These devices are usually based upon the registration, upon a kymograph, of the redistribution of weight of a suspended system, of which the animal under experimentation represents the most important part. In its earliest form[58] the platform upon which the animal lay was so adjusted that one end of it rested on knife edges and the other end was suspended directly by a spring connecting with a tambour and kymograph. Any variation in the center of gravity of the animal moving other than vertically or in a plane precisely parallel to the knife edges changed the tension on the suspending spring, and this altered the position of the pointer writing on the kymograph. Later this system was changed, and in place of the suspension spring a pneumograph was employed or a rubber ball was placed under the suspended cage and transmitted the changes in air pressure to a sensitive tambour.

The rubber ball system obtained in the rat experiments. A rubber ball, with flexible and yet fairly rigid walls, was placed under that end of the

[57] The high quotients noted by Wesson (*Jour. Biol. Chem.*, 1927, LXXIII, 449) even after 72 hours of fasting we have never experienced.

[58] Benedict, F. G., and J. Homans, *Jour. Med. Res.*, 1912, XXV, 426.

rat cage suspended by the spiral spring (see Figure 1) and was connected by small rubber tubing to a tambour with pointer. This pointer lightly touched the smoked paper on a slowly revolving kymograph drum. Since the cage was elongated, any body movement of the rat would in practically all cases be in a direction not parallel to the knife edges, and hence slight shifts in the center of gravity would usually be recorded at once. During an experiment the tracing on the kymograph drum was, for the most part, a fairly straight line, broken by minor movements of the animal, the degree of displacement of the pointer being roughly proportional to the magnitude of the body movement and the number of breaks in the line equal to the number of body movements.

Two points of technique developed immediately in the use of these kymograph tracings. In the first place, a straight line *without any change* could be interpreted as a real picture of complete muscular repose only when confirmed by ocular observation, for it was not impossible that some friction on the cage or a poorly made connection would cause the apparatus to fail to register. This difficulty could usually be discovered by dropping a weight of 10 to 15 grams upon the cage before the experiment began and noting the displacement of the tambour pointer. Usually this test of sensitivity was applied at the end of the experiment likewise. Occasionally, however, after the glass cover was put in place or in the very operation of putting the cover in place some displacement of the complex system might be made. Hence if a straight line was obtained without a break, some error in the technique was suspected. Indeed, if the animal was seen to move and there was no graphic registration of activity, this was considered proof positive of defective technique. This latter test is feasible with respiration chambers such as the glass-covered chamber for rats, in which the animal is visible.

But the most important feature of this technique lies in the interpretation of the kymograph curves. In our earlier experiments with children an attempt was made to integrate the movements of the cage by a work-adder device,[59] but this was only partially successful. Hence in the experiments with rats the interpretation of the curves was confined to an estimate of the gradations in the intensity of activity. *Before any inspection of the metabolism measurements*, a rough quantitative estimate of the activity represented by each curve was made by assigning to the curve a numerical value. Thus, a straight line with few deviations was assigned

[59] Benedict, F. G., and F. B. Talbot, *Carnegie Inst. Wash. Pub.* No. 201, 1924, Fig. 8, p. 60: *ibid.*, *Amer. Jour. Dis. Child.*, 1914, VIII, 26.

the value I, representing the minimum activity. A line with numerous and wide fluctuations was given the value III, signifying that the activity was continuous or great. Activity II was intermediate. Admittedly this classification must be crude.

As an indication of the type of activity records obtained in these experiments and as an answer to the numerous questions which have been received at the Nutrition Laboratory regarding the interpretation of these records, typical curves are reproduced in Figure 3. The top record illustrates the activity of a rat, which has been interpreted as representing on the average for the entire experiment activity III, although there are several periods when the animal was motionless. Activity II is typified by the curve shown in the center of Figure 3. For the most part the rat was quiet, but there were a number of small movements throughout the period of measurement. The minimum activity, or activity I, is illustrated by the lowest curve in Figure 3. Even in this curve, however, there are slight deviations in the line, deviations which are really necessary to demonstrate that the registering device is functioning properly. Obviously the magnitude of the deviations from the straight line will be determined in large part by the length of the tambour pointer and the arrangement of the lever and fulcrum. In most of our work the total length of the pointer was about 21 cm., and the fulcrum was placed from 3 to 5 cm. from the bearing end of the pointer. Although these curves here illustrated do not show records of sensitivity tests, it has been the custom to write the sensitivity test on the record both at the beginning and at the end of each day's work.

Relation Between Cage Activity And Metabolism

With humans, particularly with children,[60] it was soon seen that this record of activity was reflected immediately in the metabolism measurements. With cooperative adults, however, in short experiments the deviations of the pointer were rarely sufficient to justify including the graphic registration of activity as a part of the regular laboratory technique. With animals the influence of variations in the degree of repose upon the metabolism was strikingly brought out by Benedict and Homans.[61] In the case of a dog lying on a suspended cage the amount of activity, as shown by the graphic tracings, was indirectly proportional to the rise in environmental temperature. At a temperature of about 14° C. the

[60] Benedict, F. G., and F. B. Talbot, *Carnegie Inst. Wash. Pub.* No. 201, 1914, 130 et seq.

[61] Benedict, F. G., and J. Homans, *Amer. Jour. Physiol.*, 1911, xxviii, 40; *ibid.*, *Jour. Med. Res.*, 1912, xxv, 434 *et seq.*

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[61] Benedict, F. G., and J. Homans, *Amer. Jour. Physiol.*, 1911, xxviii, 40; *ibid.*, *Jour. Med. Res.*, 1912, xxv, 434 *et seq.*

activity was greatest. With each elevation of temperature it regularly decreased until at 23° C. no activity was noted. The lower temperatures were likewise accompanied by increased metabolism. On the basis of a

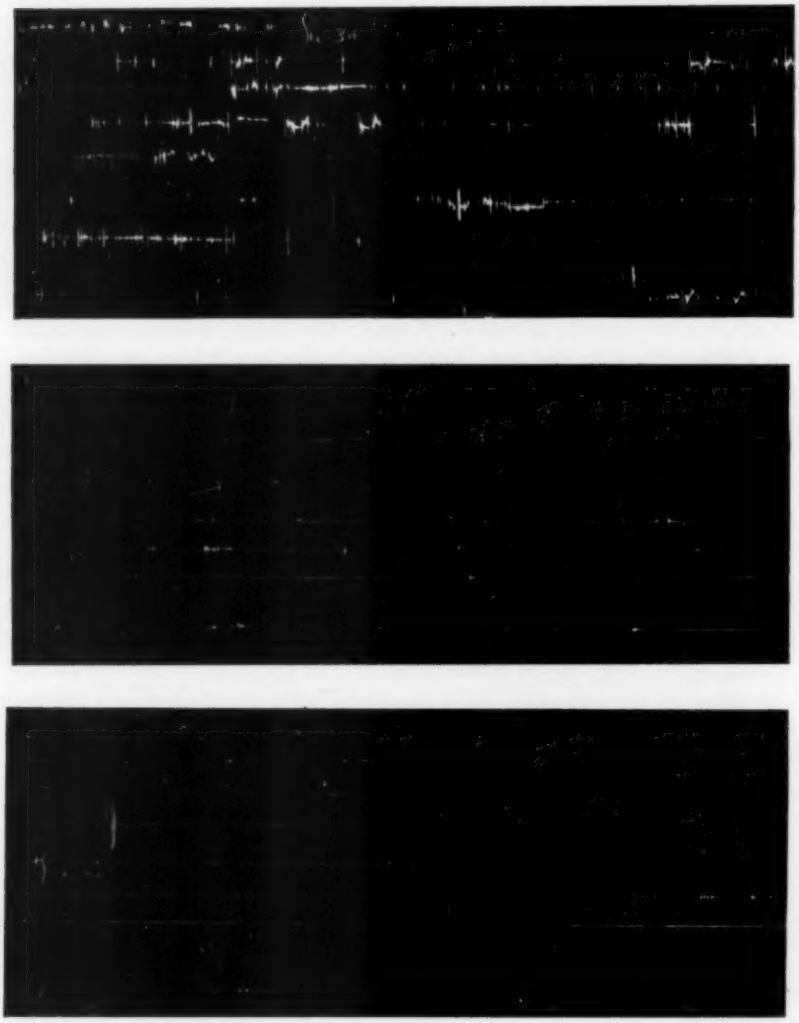

FIG. 3.—Typical kymograph records of the degree of activity of the rat during metabolism measurements.

The top curve is typical of excessive activity III, the middle curve of moderate activity II, and the bottom curve of minimum activity I.

finding such as this, emphasis was laid, perhaps unduly, upon the significance of muscular movements as explanatory of the well-known increase in metabolism obtaining at low temperatures.

This same principle of registering changes in the center of gravity of the body of the experimental animal was actually employed with 600-kg. steers at Durham, New Hampshire, and usually successful records were obtained. It was soon found with steers that a rather considerable amount of so-called "activity," such as the swaying of the body from one side to the other, was not accompanied by a large change in the metabolism. Indeed, unless the steer was unusually heavy and extremely restless, the "stall activity" rarely increased the metabolism more than 15 per cent above the minimum metabolism.[62] We therefore began to suspect that undue stress had been laid by us upon the registration and significance of activity, at least with animals and certainly so far as its bearing upon many physiological questions is concerned.

With the activity records at hand for the albino rat an analysis can also be made of the relationship between the metabolism and the activity during periods of minimum and maximum movement. Since physiologists have repeatedly asked for further details concerning our experience with graphic activity records, it seems best to make this analysis first, before discussing the influence of other factors upon the rat's metabolism.

With the rat, as with the dog, when the environmental temperature was low the activity was marked and there was an increased metabolism. Thus, at a temperature of 31° C. the heat production of a rat, 16 hours after food, was 17.6 calories per 200 grams of body weight per 24 hours. The same rat at a temperature of 13° C. and again 16 hours after food had a heat production of 48.6 calories, or 176 per cent above that at 31° C. In this instance, as in many other similar comparisons, the kymograph curves showed that the activity was greater during the cold experiment than during the warm. The question naturally arises as to how much of this increase in metabolism is to be attributed to physical activity, shivering and body movements, and how much to the true chemical regulation of heat production. In contrast to the experiments showing increased metabolism with increased activity at the lower temperatures, many duplicate experiments made at the same temperature, whether high or low, showed that there might be activity I in one test and activity II or III in the duplicate test, and yet the metabolism would be essentially the same in both instances. From this preliminary inspection of the data,

[62] Benedict, F. G., and E. G. Ritzman, *Carnegie Inst. Wash. Pub.* No. 324, 1923, 209; *ibid.*, *Carnegie Inst. Wash. Pub.* No. 377, 1927, 229.

therefore, it was difficult to note any pronounced influence of activity *per se* upon the metabolism. Certainly the moderate differences in metabolism resulting from considerable differences in activity were insignificant when compared with the profound differences in metabolism produced by the changes in environmental temperature.

These experiments with great changes in environmental temperature were not ideal for studying the effect of variations in muscular activity. Moreover in these experiments only the average activity and the average metabolism for the entire experiment were compared. Therefore direct comparison was next made of the activity and the metabolism in the individual, successive periods of any one experiment. In such cases the environmental temperature was constant throughout all the periods and the influence of this factor was ruled out. The relatively few instances in which extreme differences in activity occurred from period to period were first considered. In the majority of these the highest metabolism was found in the periods with the greatest activity, but the conditions were often reversed and the highest metabolism occurred in the periods of least activity. Comparison of period values, however, is not entirely satisfactory, because of the potentiality of error in the carbon-dioxide measurement in any single period. Both the oxygen consumption and the carbon-dioxide production were determined in these experiments with rats, but the carbon-dioxide measurement has been used as an index of the metabolism since, theoretically, it should be the more accurate, being least influenced by changes in temperature or atmospheric pressure. The result for each period, however, depends upon individual weighings and not upon duplicate analyses, and hence such a result does not have the specific value of a carefully controlled figure. Hence it is only from the general picture of all the data that one can safely draw any conclusions.

A comparison of the activity and the metabolism has also been made in experiments in which there was great uniformity in the carbon-dioxide measurements from period to period. The activity for each of these periods was estimated without reference to the metabolism values, and then the degree of irregularity in activity under conditions of uniform metabolism was noted. The data available for this comparison indicate that in general, when the metabolism was essentially the same from period to period on any one day, the activity likewise remained unchanged, although there were a few instances in which the activity increased from I to II or from II to III.

The relation between cage activity and metabolism may be studied in many ways. Thus, a comparison may also be made in experiments in which the activity is uniform from period to period but in which there

are maximum differences in metabolism. It then becomes a problem to determine the magnitude of the difference in metabolism caused by difference in activity, i.e., the mathematical difference between the metabolism measured in a period with activity I and that measured in a period with activity III. A careful examination of all the data on this point convinces us (1) that there is nothing to alter the general thesis that the ideal experimental periods are those with complete muscular repose, (2) that a minor amount of muscular activity is in general without effect on the metabolism, (3) that momentary, excessive activity seemingly has only a minor influence in a 2-hour period, but (4) that repeated, intermittent activity undoubtedly makes for an increase in metabolism which may amount on the average to not over 15 or 20 per cent, but which may, if the activity is long continued and persistent, considerably exceed this.[63]

No instances were found in which the increase in metabolism due to activity has approximated that recorded by Bijlsma,[64] i.e., 50 per cent. Bijlsma's extremely ingenious apparatus, however, is designed only for short periods of a few minutes, and hence continuous activity in such a case would produce the maximum effect. Our periods were rarely less than two hours in length, and the length of period naturally minimizes the effect of any momentary muscular activity. Furthermore, the after-effect of muscular activity would play a relatively small role in long periods of measurement, whereas it might play a considerable role in short periods. For certain comparisons short periods of measurement are suitable. We doubt if the basal metabolism of the rat can be as satisfactorily studied in a short as in a long period, unless it is possible to secure a short period with complete muscular repose, preceded by repose of fairly long duration. This condition apparently was met for the most part in the experiments of Mitchell[65] who, although measuring the metabolism in periods nearly twice as long as those of Bijlsma, nevertheless states that during this length of time his rats remained quiet and undisturbed.

The general conclusion is that in practically all cases the graphic record of activity can be dispensed with, especially if it is possible to observe the animal continually. Even when there is considerable movement, the actual increase in metabolism may be hardly more than 15 per cent. Yet, an animal which is moving about considerably is not a suitable one for studying the influence upon metabolism of subtle, superimposed factors, and one should always remember that in short periods it is possible for the metabolism to be increased by activity from 15 to 50 per cent.

[63] See also Lee, M. O., and E. F. Van Buskirk, *Amer. Jour. Physiol.*, 1928, LXXXIV, 329.
[64] Bijlsma, U. G., *Verslagen en Mededeelingen betreffende de Volksgezondheid*, August, 1926.
[65] Mitchell, H. H., and G. G. Carman, *Amer. Jour. Physiol.*, 1926, LXXVI, 388.

In the case of the albino rat, we are, however, inclined to withdraw our earlier emphasis (which was admittedly too strong) upon the imperative necessity for graphic registration of the activity, cautioning that the animal must always be visible and that if it is seen to be continually or considerably active, the results of the experiment should not be used for studying the minor influences of superimposed factors. Certainly if one avoids the use of active rats, the registration of activity, in so far as it serves simply as an index of whether the conditions of repose have been ideal for basal metabolism measurements, is perhaps in general an unnecessary complication. Relinquishing the graphic records does, without doubt, make less sure the suitability of a given animal for the test under consideration. It hardly seems necessary to train a rat for a period of two weeks, as suggested by Arnoldi[44] and yet for the study of many subtle factors the possible necessity for such training must always be held in mind.

In the analysis of the data presented in this paper, since activity records were secured in *all* of the experiments, they have been taken into consideration and, as will be seen in the subsequent discussion, these records have contributed materially to the interpretation of results, particularly in the study of the influence of a number of rather subtle factors.

Method of Presenting Results

Since in these experiments the measurements were not of the heat production directly but of the carbon dioxide exhaled and the oxygen consumed, the heat production is calculated indirectly. Shortly after the beginning of our research it seemed clear that with rats the respiratory quotient is almost invariably not far from 0.72 or 0.73 when food has been withheld for 16 hours, and that immediately after food the quotients are higher, as is to be expected. Most of our experiments were made not less than 16 hours after food. In general, therefore, practically all of the heat values reported by us are based upon the measurement of the carbon-dioxide production and a determined respiratory quotient very close to 0.72.

The total heat production can be referred either to body weight or to body surface. Since many of the first experiments were made with rats averaging not far from 200 grams in weight, the heat production was first computed per 200 grams of body weight. When the influence of age was introduced, rats of smaller body weights were studied, and it then seemed best to refer the heat production to some power of the body weight. It has long been the custom of physiologists to calculate the body surface

[44] Arnoldi, W., *Zeitschr. f.d.ges. exp. Med.*, 1926, LII, 258.

of various animals from the two-thirds power of the weight, multiplied by a constant. In the case of the albino rat the constant of 9.1 originally applied by Rubner[67] has been generally used, although A. V. and A. M. Hill[68] in 1913 concluded from actual measurements of the surface area that the constant is essentially 10. In 1925 Giaja[69] proposed the constant 8.99, based upon his own measurements, and in 1926 Carman and Mitchell[70] suggested a value of 11.36. The method of measurement employed by Carman and Mitchell is distinctly at fault, in that they admittedly stretched the skins of their rats, and the area measured was therefore large. The more recent, unpublished technique of Lee[71] gives results indicating an average constant of not far from 9.2, strongly confirming the original constant of Rubner, which it is interesting to note has not been bettered after forty years. Inasmuch as the rat has a loose skin and inasmuch as Rubner[72] states expressly that skin area is not synonymous with radiation area, one cannot feel secure in using any of these constants. In all probability the surface area cannot be less than that obtained by the use of the Rubner constant, and the constants of the Hills and especially of Carman and Mitchell are probably too high. After a most careful survey of the literature and particularly after a critical analysis of the various techniques, we have seen no reason to alter the value of the constant given by Rubner for the rat, namely, 9.1, and since, according to the results of Carman and Mitchell, the constant for small rats is not materially different from that for large rats, we have calculated the body surface for *all* our rats, no matter what their size, by the formula $S = 9.1 \times W^{2/3}$, in which S equals the surface in square centimeters and W the weight in grams.

The suggestion of Cowgill and Drabkin[73] that a body length measurement be included in estimating the surface area of animals came too late for application in our study. It is the experience of others, however, that this length measurement is difficult to secure unless the animals are at

[67] Rubner, M., Die Gesetze des Energieverbrauchs bei der Ernährung, Leipzig, 1902, 281.

[68] A. V. and A. M. Hill, *Jour. Physiol.*, 1913, XLVI, 95.

[69] Giaja, J., *Ann. de Physiol.*, 1925, I, 607.

[70] Carman, G. G., and H. H. Mitchell, *Amer. Jour. Physiol.*, 1926, LXXVI, 380.

[71] After our research was finished and the calculations were completed, Dr. Milton O. Lee of the Harvard Medical School kindly informed us in a private communication that by an entirely different and to us extremely novel method he has been measuring the surface area of rats and finds constants which are 19 per cent below the constant of Carman and Mitchell. Hence Lee's results confirm those of Rubner and Giaja and are about 10 per cent lower than the constant used by A. V. and A. M. Hill.

[72] Rubner, M., Beiträge zur Ernährung im Knabenalter mit besonderer Berücksichtigung der Fettsucht, Berlin, 1902, 40.

[73] Cowgill, G. R., and D. L. Drabkin, *Amer. Jour. Physiol.*, 1927, LXXXI, 36.

least partly anesthetized. Whether a body length measurement will be generally incorporated in the surface formula as being a betterment of the Meeh formula with the Rubner constant is still to be demonstrated.[74]

In much of the discussion in this and the following paper (see note 1, p. 366) body surface, as such, plays no role. Thus, analyses of the effect of environmental temperature upon the same rat, of the effect of age after full growth has been attained, the effect of sex (in which rats of the same body weight are compared), or the effects of various superimposed factors such as special diets and pregnancy, do not involve serious consideration of the surface area. On the other hand, surface area does play a role in the comparison of extremely small rats with more mature rats or with large adult rats, and above all in comparative physiology, when the heat production of the rat is compared with that of other animals. In the presentation of our results restriction of space has necessitated giving the metabolism data on only one basis of measurement. We have therefore chosen to report the heat production per square meter of surface area per 24 hours, expressed as large calories. Since the weight of the rat is also usually given, it is feasible to compute from these data the heat production per 200 grams of body weight, i.e., per unit of weight.

Discussion of Results

Influence of Fasting

The ingestion of food increases the metabolism. With man it is assumed that the effect of food ingestion has passed after 12 hours, provided that the preceding meal has not been too rich in protein. With dogs a 24-hour fast is commonly considered the prerequisite for basal metabolism measurements. This 24-hour fasting has likewise been the custom in many laboratories in the study of small animals such as rats, birds, or guinea pigs. With ruminants, particularly large steers, the stimulating effect of food is found in some instances to continue for perhaps 12 hours longer and the metabolism does not reach its basal level until about 36 hours after food.[75] It was therefore important to study the influence of fasting upon the metabolism of the rat, in order to note particularly at what point the increment in metabolism due to digestive activity ceases.

In this study several groups of experiments contribute to the solution of this problem. For example, twenty rats were studied immediately

[74] See, also, the treatment of this point given by S. Brody, Univ. Missouri, *Agric. Expt. Sta., Bulletin* 115, 1928.

[75] Benedict, F. G. and E. G. Ritzman, *Carnegie Institution Wash. Pub.* No. 377, 1927, 208.

after food was taken away from them and again 24 hours later. Fifty-five rats were studied 17 hours and also 42 hours after food, and twenty-two of these same rats were studied not only 17 and 42 hours but likewise 64 hours after eating. The average results of these experiments are reported in Table I, expressed as calories per square meter of body surface per 24 hours. Since these fasting studies represented a stage of development in our research, the chamber temperature was not the same in all experiments. Nevertheless the average temperature at which all of these groups were studied was for the most part 26° C., so that in analyzing the average results for the different groups the temperatures can be considered comparable. There was no means of knowing how recently the rat had eaten up to the moment when food was taken away, and hence

TABLE I

HEAT PRODUCTION OF THE FASTING RAT AT 26° C.

Number of rats in group	Hours after food	Heat produced per sq. m. per 24 hrs.
		Cal.
	0	1065
20	24	929
	17	993
22	42	1012
	64	976
	17	1029
55	42	1016

considerable variability in the heat production of individual rats during the period of measurement supposedly immediately after the removal of food is naturally to be expected. In the group of twenty rats most were over 16 months old, although six were 4 1/2 months old or under. In the larger group of fifty-five rats practically all were between 17 and 24 months of age; five were five months of age or younger.

Without doubt the differences in age and the slight differences in environmental temperature affect these comparisons, but the general picture is that immediately after withdrawal of food the average heat production is 1065 calories per square meter of body surface per 24 hours, 17 hours afterwards it is 993 calories, 24 hours after food it is 929 calories, 42 hours after food 1012 calories, and 64 hours after food 976 calories.[78] Thus the highest heat production occurs, as is to be expected, immediately

[78] Although we are dealing here with the metabolism of small animals, for purposes of comparison the 24-hour heat production is given in large calories per square meter of body surface as this is the custom followed in the reports of measurements of all other animals.

after food, and the metabolism falls off 72 calories at the seventeenth hour, with a further marked fall at the twenty-fourth hour. The comparison, however, should more correctly be based upon the data for the same group of animals, in which case the change in metabolism is known not for the first 17 hours after food but only for a longer period of 24 hours, during which time the decrease in heat production amounts to 136 calories. It is perhaps surprising that the fall in metabolism (7 per cent) is not greater in 17 hours, although this is in line with the contention of Mitchell and Carman[77] that the so-called "specific dynamic action" of food, when the diet is sufficient for maintenance, is probably inappreciable. There is seemingly a fall of 6 per cent between the seventeenth and the twenty-fourth hours, but if one compares the same group of animals at the seventeenth and the forty-second hours, there is no significant change.

The most striking feature of this study is the fact that the metabolism remains so constant after 17 hours.[78] It would therefore appear as if, so far as cessation of digestive activity is concerned, a fasting period of 17 hours is ample to bring the rat to a low, steady level of metabolism. The effect of any superimposed factor can thus apparently be satisfactorily studied after the rat has been without food for 17 hours.

Further studies with fasting rats are needed to make certain points clearer. Thus, since it was difficult to determine the exact time when food was last eaten by these rats, it is possible that in the period of measurement made immediately after the withholding of food some of the rats were studied at once after eating and others possibly well over into the 17-hour period. Our protocols do not contain sufficient comparable data with any one rat to show clearly any difference in metabolism during this time. The change in metabolism due to fasting should therefore be studied further with individual rats in experiments in which the metabolism after the withholding of food is continually observed from short period to short period. Such observations should prove whether with rats the metabolic level corresponding to the basal metabolism of man is reached in a period of fasting somewhat shorter than 17 hours, a question not answered by our results. Experiments with fasting periods longer than 64 hours are likewise desirable, for although the average heat production of twenty-two rats which fasted 64 hours was not appreciably lower than it was when they had fasted only 17 hours, it has not yet been shown that more prolonged fasting would not result in a still lower metabolism.

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[77] Mitchell, H. H. and G. G. Carman, *Amer. Jour. Physiol.*, 1926, LXXVI, 410.

[78] See, also, A. V. and A. M. Hill, *Jour. Physiol.*, 1913, XLVI, 96.

The temperature at which these rats were studied, 26° C., is somewhat lower than the critical temperature for the rat. It is conceivable that, since the rat's metabolism is more rapid at the lower environmental temperatures, (see note 1, p. 366) a 24-hour period without food at the lower temperatures would represent a much more advanced stage of fasting than would the same length of fasting at the higher temperatures. It is still to be demonstrated experimentally that the metabolism will not be lowered more by a fast of 24 hours at 21° C., for example, than it will by a fast of 24 hours at 26° C. In other words, the increase in metabolism due to lower environmental temperatures may be used as a factor to intensify the effect of fasting, and fasting experiments at lower temperatures should add much to our information on this important point.

If the general thesis is correct that the metabolism of these small animals is extremely intense, one would expect that with the rat a fasting period of 64 hours would correspond to a fast of many days with man and that the metabolism 64 hours after food would be much lower than that obtaining immediately after food. Our finding that even after 64 hours of fasting the metabolism is essentially the same as it was 17 hours after food is therefore astonishing. This sustained level of metabolism with continued fasting cannot be explained by increased activity. One might conceive that the hunger urge makes for a greater degree of restlessness and activity. But the kymograph records show that the activity during these prolonged fasting periods was not increased. The constancy in the metabolism between the seventeenth and the sixty-fourth hours of fasting might be considered as an argument that the body stores of these small animals are rapidly depleted by fasting and that at this stage of fasting the rat's metabolism has reached the level noted with man and with the steer only after a number of days of fasting. Against this argument is the fact that with man and with the steer the difference between the basal metabolism and the metabolism at the prolonged fasting level is very great, *circa* 30 per cent. With a group of twenty rats, on the contrary, the average heat production per square meter of body surface per 24 hours decreased from 1065 calories immediately after food to 929 calories 24 hours later, i.e., a decrease of 13 per cent. With another group of fifty-five rats there was no change between the seventeenth and the forty-second hours. Hence we can conclude that with rats the total decrease in metabolism to be expected between the time when the last food is eaten and the time when this sustained lower level is reached is but 13 per cent, or only one-half that noted with humans and steers.

Of the twenty rats with which, during the first 24 hours of fasting, an average lowering in metabolism of 13 per cent was noted, six were young

rats 4 1/2 months of age or under. The metabolism of these young rats during the 24 hours of fasting fell off 28 per cent, thus emphasizing that the young organism is the least fitted to withstand fasting. Therefore, although a period of 17 hours without food will undoubtedly be necessary with rats 5 months old or over before cessation of digestive activity occurs and the metabolism can be considered to be basal, it is more than likely that by the seventeenth hour very young rats are much farther along in the stage of fasting, as is shown by the decrease in their metabolism at the end of the twenty-fourth hour.

Summary

A closed-circuit respiration chamber for measuring (usually in 2-hour periods) both the carbon-dioxide production and the oxygen consumption of the rat is described, and the methods employed by other investigators in studying the gaseous metabolism of the rat are listed.

Description of the method of graphic registration of the rat's activity and discussion of the quantitative interpretation of such records is followed by a study of the relation between the rat's metabolism and its activity. It is concluded that the ideal experiments are those with complete muscular repose, that in 2-hour periods a minor amount of muscular activity is in general without effect upon the metabolism, that momentary excessive activity has only a minor influence in a 2-hour period, and that repeated, intermittent activity, if not long continued, causes an increase in metabolism of not over 15 to 20 per cent. The necessity for graphic registration of activity as an index of whether the conditions of repose have been ideal for basal metabolism measurements has therefore been over-emphasized in the case of the rat. In general, ocular observation of the activity and discarding of results obtained when the rat is seen to be constantly moving (especially during the study of the influence of a subtle, superimposed factor) will be sufficient.

The heat production of rats over 4 1/2 months old (reported as large calories per square meter of body surface per 24 hours), measured at 26° C., decreased on the average from 7 to 13 per cent during the first 17 to 24 hours without food, but remained essentially constant thereafter up to the sixty-fourth hour. The effect of any superimposed factor can thus be satisfactorily studied after the rat has been fasting 17 hours. With rats younger than 4 months the metabolism decreased 28 per cent during the first 24 hours of fasting. The younger organism is therefore not so well able to withstand fasting.[1]

[1] A second article on the heat production of the albino rat by the same authors will appear in the May number of this Journal.—Ed.